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STUDIES OF THE ROOT NODULE ORGANISMS OF CERTAIN WILD LEGUMES[1]

MARIE ECKHARDT CONKLIN[2]

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The present investigation is concerned with a study of the root nodule bacteria of certain legumes which grow wild in Massachusetts, Connecticut, and New York and which may have an importance in natural green-manuring far greater than has been credited to them. Its purpose is to show, by a study of their morphological, cultural, and physiological characteristics, the relationship between the organisms isolated from different wild legumes, to characterize them so that they may be recognized by definite reactions, to establish their cross-inoculation position, and to show their relationship to other members of the inoculation group to which they may be found to belong.

The organisms were isolated from the following plants, the names, with two exceptions (6, 7), being taken from Gray's Manual (1908)[3]: *Amphicarpa bracteata* (L.) Fernald [*A. monoica* (L.) Ell.], *Baptisia tinctoria* (L.) R. Br., *Cassia nictitans* L., *Crotalaria sagittalis* L., *Desmodium paniculatum* (L.) DC., *Genista tinctoria* L., *Lathyrus latifolius* L., *Lathyrus japonicus*, Willd., var. *pellitus* [*L. maritimus* (L.) Bigel.], *Lespedeza hirta* (L.) Hornem., and *Lespedeza frutescens* (L.). Most of these plants are widely distributed in the eastern part of the United States. Although *Lathyrus latifolius* is not a native species, it is, according to Gray's Manual (1908), "frequently cultivated, and escaping to roadsides and thickets, Ct. to D. C." In addition, *Dolichos Lablab* L., an introduced form which is cultivated in Japan as a legume crop [Akemine (1931)], was used as a source of two cultures.

HISTORICAL

Scattered references to the characteristics and cross-inoculation position of some of the wild legume organisms described in this paper occur in the literature and are reviewed in the monograph on the root nodule bacteria by Fred, Baldwin, and McCoy (8). The principal conclusions drawn from these previous studies are that *Amphicarpa bracteata* will not cross-

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[1] Brooklyn Botanic Garden Contribution No. 73.

[2] The writer takes pleasure in acknowledging her indebtedness to Dr. E. B. Fred and Dr. I. L. Baldwin, of the University of Wisconsin, for their helpful suggestions concerning this investigation; to Dr. L. M. Snow, of Wellesley College, for making part of this work possible; to Dr. S. F. Trelease, of Columbia University, for careful criticism of the manuscript; and particularly to Dr. G. M. Reed, of the Brooklyn Botanic Garden, under whose guidance the major portion of the work was carried out.

[3] See note to "references."

inoculate with any other common legume; that *Baptisia tinctoria*, *Cassia nictitans*, and *Genista tinctoria*, on the basis of studies made with one strain of organism from each host, belong to the cowpea group, as do certain species of Crotalaria, Desmodium, Lespedeza, and possibly *Dolichos Lablab*; and that some species of Lathyrus belong in the pea group. The monotrichous flagellation, slow growth on ash agar, and inability to produce marked changes on litmus milk or to liquefy gelatin which have been noted for the organisms from *Baptisia tinctoria*, *Genista tinctoria*, and certain species of Amphicarpa, Cassia, Desmodium, and Lespedeza, and the peritrichous flagellation, rapid abundant growth on ash agar, and ability to change litmus milk which have been noted for the strains from certain species of Lathyrus are the chief physiological characteristics so far assigned to these organisms. Recently, Carroll (5) has placed *Desmodium triflora*, *Lespedeza sericea* and *L. stipulaceae* in the cowpea inoculation group, and *Lathyrus tingitanus* in the pea group and has noted fairly close protein kinship among fifteen species of Crotalaria and, in most cases, between the Crotalaria species and cowpea.

MATERIAL AND METHODS

Cultural study

Cultures were isolated from plants which Dr. Alice M. Ottley, of the Wellesley College Botany Department, and Dr. H. K. Svenson, of the Brooklyn Botanic Garden, very kindly identified.

Stock cultures were carried on yeast-extract mannitol agar to which Congo red, when making isolations, and brom thymol blue, when required to indicate hydrogen-ion concentration (9), were added.

Litmus milk, Endo's agar, and lead acetate agar were prepared from the "Difco" dehydrated product of Digestive Ferments Co., the milk being sterilized 10 minutes at 8 pounds' pressure for 3 consecutive days, and incubated for 1 week at 28°C. to test for sterility before using. Potato slants (9), neutral red glycerol ammonium phosphate solution (11), asparagine tyrosine agar made by adding 1.5 gm. of tyrosine to 1,000 cc. of asparagine mannitol medium (9), and standard beef-extract peptone gelatin sterilized for 10 minutes at 8 pounds' pressure on three consecutive days were also used.

Sugar media, with brom thymol blue indicator [Baldwin and Fred (1927)], were prepared from arabinose, glucose, fructose, lactose, maltose, sucrose, or raffinose and sterilized as in previous studies [Eckhardt, Baldwin, and Fred (1931)]. By the use of a standard method of sterilization, the results of the fermentations are comparable in spite of the slight destruction of certain sugars by heat. To discover the extent of this fixed error, growth and reaction on fructose agar prepared in the foregoing manner and prepared by adding the sugar solution, sterilized through a Berkefeld filter, to the previously sterilized nitrate agar were compared. In most cases growth was the same; in a few cases, slightly more abundant on the medium sterilized by filtration. The two media varied only slightly in their final fermentation reaction.

Dextrin; starch; the glucosides amygdalin, aesculin, and salicin; the alcohols dulcitol, erythritol, ethylene glycol, and glycerol; and the organic acid salts potassium oxalate, sodium succinate, and sodium urate were also used in 1 per cent concentration in the aforementioned basic medium. The fer-

mentation reactions produced by the organisms on the carbohydrate media were recorded as pH values for comparative purposes, but in interpreting these results, the limitations of the method must be considered.

Representative amino acids as the sole carbon and nitrogen supply were added to a mineral solution of the following composition:

Dibasic potassium phosphate	0.5 gm.
Magnesium sulfate	0.2 gm.
Sodium chloride	0.2 gm.
Distilled water	1,000.0 cc.
Brom thymol blue, 0.5 per cent alcoholic solution	5.0 cc.

To tubes containing the sterilized solution, 0.5 per cent alanine, cystine, leucine, tyrosine, or a mixture of these four, previously sterilized by filtration, or in the dry state at 15 pounds' pressure for 15 minutes, was added under sterile conditions. Since cystine and tyrosine are difficultly soluble in water, the precipitate made judgment of growth impossible, and observations of reaction changes were used in these cases to compare the growth activities of the organisms, although absence of any change did not necessarily indicate failure to grow. Other media containing 0.5 per cent alanine, leucine, or tyrosine, and 1.5 per cent agar to permit more accurate comparison of growth were prepared. A growth accessory factor isolated from cane sugar of retail commerce by the method of Allison and Hoover (1) was added to three sets of the amino acid agar in concentration of 0.008 per cent; of these, one set also contained 1.0 per cent mannitol, and a second, 0.05 per cent potassium nitrate.

Dye and salt sensitivity were tested by the plate streak method, using nitrate mannitol agar to which had been added varying concentrations of dibrom-oxymercuri fluorescein, or in which the amounts of sodium chloride, magnesium sulfate, and dibasic potassium phosphate were varied. Resulting growth was in each case compared with that of a control.

All cultures were incubated at 28°C., and transfers were always made from stock cultures. Many isolations were made from each host plant in an attempt to avoid generalizations without due consideration of possible strain variations.

Microscopic study

Gram or dilute carbol fuchsin stains were made from cultures 24 hours, 1 week, and 1 month old. Flagella stains (12) were made after transferring 24-hour agar streaks to sterile water and incubating for 3 days.

Plant inoculation

Bacteria-free seeds, often filed with a sterile file to hasten germination and then washed in mercuric chloride followed by sterile distilled water, were germinated and planted in sterile washed sea sand (found by repeated Kjeldahl determinations, modified to include nitrates, to be free from nitrogen) in

glazed or paraffin-coated jars or in Erlenmeyer flasks. Modified nitrogen-free Shive's or other standard nutrient solution was sterilized and used as needed. Contamination was guarded against by growing most of the plants in sterile glass cases or covering the pots with cellophane or ground cork and watering through a sterile tube. Six weeks' or 3 months' growth was required by many of the slow-growing wild legumes. Controls, uninoculated, were distributed among the inoculated plants and found to be nodule-free.

EXPERIMENTAL

All cultures were isolated and studied by the writer working at Wellesley College and Brooklyn Botanic Garden except those from *Lathyrus latifolius*, which were isolated and studied by Miss Lydia Kittell, under the direction of Dr. L. M. Snow at Wellesley College.

Since it was found, after cultural and cross-inoculation studies were made, that the organisms under investigation fell into two main groups, a characterization under this grouping follows. Due consideration should be given to the strain variations which are noted in the detailed tabulations.

Group 1 includes 20 cultures isolated from *Amphicarpa bracteata*, 24 from *Baptisia tinctoria*, 27 from *Cassia nictitans*, 5 from *Crotalaria sagittalis*, 10 from *Desmodium paniculatum*, 9 from *Genista tinctoria*, 3 from *Lespedeza hirta*, 2 from *L. frutescens*, and 2 from *Dolichos Lablab*. Group 2 includes 3 cultures isolated from *Lathyrus latifolius*, and 13 from *L. japonicus*.

Characterization of Group 1

The organisms of group 1 are fine, Gram-negative rods, bearing at the "corner" a single flagellum, and often swollen, vacuolated, and slightly irregular with age. On yeast-water mannitol agar they show rather slow, scant to moderate, white or colorless, smooth growth, without absorption of Congo red, and with an alkaline fermentation of mannitol, no growth or very scant white or creamy growth on potato slants, with substrate unchanged, and no visible change of litmus milk beyond an alkaline reaction in some cases after 2 weeks' to a month's incubation. No visible change is produced on neutral red glycerol ammonium phosphate solution; growth on gelatin is poor or lacking with no liquefaction within three months; no appreciable amount of tyrosinase is formed, although growth on asparagine tyrosine agar is good.

Growth of representative strains from Amphicarpa, Baptisia, Cassia, Desmodium, and *Lespedeza hirta* is fair on Endo's agar, and small amounts of aldehydes are formed; usually negligible amounts of H_2S are produced on lead acetate agar. Growth is inhibited by 1.0 to 2.0 per cent dibrom-oxymercuri fluorescein agar, sensitivity generally decreasing with 0.5, 0.1, 0.05, 0.025, and 0.01 per cent, and normal or increased growth resulting from the presence of 0.005 per cent of the dye. Complete inhibition of growth of most strains with 0.5, 1.0, and 2.0 per cent K_2HPO_4; slight or no inhibition with 0.1 per cent NaCl or 1.0 per cent $MgSO_4$; slight stimulation of some strains by 0.025

and 0.05 per cent NaCl or 0.1 and 0.5 per cent $MgSO_4$; and more marked stimulation by 0.5 per cent NaCl, 0.05 per cent $MgSO_4$, or 0.1 per cent K_2HPO_4 were noted on the media containing these salts.

Most strains produce an alkaline reaction on the sugar media, a few forming acid from arabinose. Arabinose, glucose, and fructose support a fair to abundant growth; lactose, maltose, sucrose, raffinose, and dextrin, a poor to moderate growth. Starch is not hydrolyzed, and growth is poor to fair. The glucosides amygdalin, aesculin, and salicin; the alcohols dulcitol, erythritol, glycerol, and glycol; and the organic acid salt sodium succinate are utilized for carbon and energy, and poor to moderate growth, usually with an alkaline fermentation, is produced. On potassium oxalate and sodium urate agars there is little or no growth. More detailed results of the fermentation tests are given in table 1.

Of the amino acids used in the media, leucine and, to a less extent, tyrosine are utilized as sources of both carbon and nitrogen by representative strains, and in most cases an alkaline fermentation of the former and a neutral or slightly acid or alkaline fermentation of the latter result. An alkaline reaction on cystine medium, complete inhibition of growth by a solution containing 0.5 per cent alanine, and impaired growth with 0.2 per cent alanine were noted. In a solution containing a mixture of alanine, cystine, leucine, and tyrosine of 0.5 per cent total concentration, far greater increase in pH occurs after growth than when any of these are present singly in 0.5 per cent concentration. A 30 to 125 per cent increase in the growth average on the amino acid media results when 0.008 per cent of an accessory growth factor is added, the percentage increase being most with alanine and least with leucine. Potassium nitrate as source of nitrogen produces little or no further increase in the average amount of growth, but mannitol as a carbon source produces an increase to an amount equal to or exceeding that of the control.

Results of all cross-inoculation tests are given in table 2. Every organism of this group produced small to medium, round or elongated nodules on the tap or lateral roots of the original host plant. Organisms from Crotalaria, *Lespedeza hirta*, and *L. frutescens* also produced nodules on cowpeas (*Vigna sinensis*), those from Amphicarpa, Baptisia, Cassia, Genista, and Dolichos on cowpeas and irregularly on soybeans (*Soja Max*), and those from Desmodium irregularly on both. Representative strains formed nodules on all other host plants of this group on which they were tested. Three strains from *Baptisia tinctoria* also formed nodules on *B. australis*; certain strains from *Genista tinctoria*, on *G. praecox* and *Cytisus hirsutus*; and the Lespedeza cultures, on *L. bicolor rosea*.

Although the foregoing characterization applies to most members of this group, variations among strains in both growth and reaction were of frequent occurrence, particularly on the carbohydrate and amino acid media. Other variations include the more capsulated, banded, slightly branched form of the Amphicarpa organisms; the slightly branched, coccoid, or club-shaped

TABLE 1

Reactions (pH) produced by cultures on carbohydrate media

CARBOHYDRATE	SOURCE AND TOTAL NUMBER OF CULTURES												
	Group 1										Group 2		
	Amphicarpa bracteata—20	*Baptisia tinctoria*—24	*Cassia nictitans*—27	*Crotalaria sagittalis*—5	*Desmodium paniculatum*—10	*Dolichos Lablab*—2	*Genista tinctoria*—9	*Lespedeza hirta*—3	*Lespedeza frutescens*—2	Variation	*Lathyrus latifolius*—3	*Lathyrus japonicus*—13	Variation
Arabinose	7.4	7.4	7.4	7.6	7.4	7.6	6.2		7.6	6.0–7.6		6.0	6.0–6.2
	(11)*	(11)	(19)	(4)	(5)	(1)	(4)		(1)			(12)	
Glucose	7.4	7.4	7.4	7.6	7.2	7.4	7.4	7.4	7.6	6.8–7.6	6.2	6.0	6.0–6.2
	(18)	(12)	(21)	(4)	(4)	(1)	(9)	(2)	(1)		(3)	(12)	
Fructose	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.2	6.8–7.6	6.6	6.2	6.0–6.6
	(19)	(12)	(23)	(4)	(8)	(1)	(5)	(3)	(1)		(2)	(12)	
Sucrose	7.0	7.2	7.2	7.4	7.4	7.4	7.4	7.0	7.4	6.4–7.6	6.2	6.2	6.0–6.2
	(15)	(15)	(14)	(5)	(7)	(1)	(6)	(1)	(2)		(3)	(9)	
Maltose	7.6	7.4	7.4	7.4	7.4	7.6	7.4	7.0	7.4	6.0–7.6	6.2	7.0	6.2–7.4
	(17)	(13)	(24)	(5)	(10)	(1)	(8)	(3)	(2)		(3)	(9)	
Lactose	7.4	7.4	7.0	7.2	7.2	7.0	7.4	7.4	7.4	6.6–7.4	6.0	6.2	6.0–6.4
	(10)	(13)	(21)	(4)	(6)	(1)	(5)	(3)	(1)		(2)	(8)	
Raffinose	7.4	7.4	7.2	7.6	7.4	7.2	7.4	7.4	7.4	7.0–7.6	7.6	7.0	6.2–7.6
	(19)	(18)	(14)	(4)	(6)	(1)	(5)	(2)	(1)		(3)	(4)	
Dextrin	7.4	7.2	7.2	7.6	7.4	7.6	7.4	7.2	6.8	6.8–7.6	7.4	7.0	7.0–7.6
	(13)	(15)	(12)	(4)	(7)	(1)	(5)	(3)	(2)		(2)	(9)	
Mannitol	7.4	7.4	7.4	7.6	7.6	7.0	7.6	7.4	7.2	6.6–7.6	6.2	6.0	6.0–6.2
	(18)	(13)	(16)	(4)	(8)	(1)	(7)	(3)	(1)		(3)	(13)	
Amygdalin	7.2	7.2	7.0	7.2	7.4	7.2	7.4		7.2	6.6–7.4		6.6	6.6
	(2)	(3)	(1)	(2)	(2)	(1)	(2)		(1)			(3)	
Aesculin	7.2	7.4	7.2	7.2	7.2	7.2	7.4		7.4	6.8–7.4		7.0	6.8–7.0
	(2)	(2)	(1)	(2)	(3)	(1)	(2)		(1)			(2)	
Salicin	7.2	7.2	7.2	7.2	7.2	7.0	7.2		7.2	6.8–7.2		6.2	6.0–6.2
	(2)	(3)	(3)	(3)	(3)	(1)	(3)		(1)			(2)	
Dulcitol	7.2	7.2	7.2	7.2	7.2	7.2	7.2		7.2	7.0–7.4		6.6	6.4–6.6
	(2)	(2)	(2)	(1)	(3)	(1)	(3)		(1)			(2)	
Erythritol	7.0	7.0	7.0	7.0	7.0	7.2	7.0		7.2	6.4–7.2		6.2	6.2–6.4
	(3)	(2)	(2)	(2)	(2)	(1)	(3)		(1)			(2)	
Glycerol	7.0	7.4	7.2	7.2	7.0	7.2	7.2		7.4	7.0–7.4		6.2	6.0–6.4
	(2)	(3)	(3)	(2)	(2)	(1)	(2)		(1)			(1)	
Glycol	7.2	7.2	7.2	7.2	7.4	7.4	7.2		7.2	6.8–7.4		7.0	7.0–7.2
	(2)	(3)	(2)	(2)	(2)	(1)	(3)		(1)			(2)	
Potassium oxalate	6.8	7.0	7.0	7.0	7.0	7.0	7.0		7.0	6.8–7.0		6.8	6.8–7.0
	(3)	(3)	(3)	(3)	(3)	(2)	(2)		(2)			(2)	
Sodium succinate	6.8	7.4	7.2	7.2	7.2	7.2	7.2		7.4	6.8–7.4		7.4	7.2–7.4
	(3)	(2)	(2)	(2)	(3)	(2)	(2)		(1)			(2)	
Sodium urate	7.0	7.0	7.2	7.0	7.0	7.2	7.0		6.8	6.8–7.2		7.2	7.0–7.2
	(2)	(2)	(2)	(3)	(2)	(1)	(2)		(2)			(2)	

* Numbers in parentheses indicate the number of strains producing the particular reaction. When the reaction was not uniform throughout the agar, the pH of the upper half of the tube was recorded. Where pH values are given as 6.0 or 7.6, they indicate an acidity of 6.0 or more and an alkalinity of 7.6 or more, since these figures represent the limits of color change of the indicator.

Desmodium organisms; the more abundant growth of the Amphicarpa and Dolichos cultures, and the more rapid growth of the Genista cultures on yeast-water mannitol agar, and the neutral or acid reaction produced by a few strains from Baptisia, Cassia, Dolichos, and *Lespedeza frutescens* on this medium; the production of a serum zone on litmus milk by one strain from *Lespedeza frutescens*, two from Amphicarpa, and two from Baptisia, reduction of the litmus by these two Baptisia cultures and by three strains from Cassia, two of which also produce an acid reaction; the formation of a limited amount of tyrosinase by a few strains from Amphicarpa and Baptisia; and an acid reaction on both tyrosine and cystine media by the Dolichos cultures.

Characterization of Group 2

The organisms of group 2 are short, often coccoid or enlarged, frequently branched, peritrichous, Gram-negative rods. They show a rapid, abundant, colorless, very gummy growth on yeast-water mannitol agar, with no absorption of Congo red, and an acid fermentation of mannitol; typical lack of or very scant, creamy growth on potato with the substrate unchanged, and a fair to moderate growth on asparagine tyrosine agar, with the production of tyrosinase negligible or entirely lacking.

All strains from *Lathyrus japonicus* produce a definitely alkaline reaction with partial to complete reduction and a half inch serum zone on litmus milk and no appreciable color change on neutral red glycerol ammonium phosphate solution. Their growth is completely inhibited by 1.0 or 2.0 per cent dibromoxymercuri fluorescein in the agar and impaired by as low as 0.005 per cent of the dye; completely inhibited by agar containing 0.5, 1.0, or 2.0 per cent K_2HPO_4; unchanged or slightly inhibited with 0.1 per cent NaCl or 1.0 and 0.05 per cent $MgSO_4$; unchanged or slightly stimulated by 0.025 and 0.05 per cent NaCl or 0.1 and 0.5 per cent $MgSO_4$; and in some cases stimulated by 0.5 per cent NaCl or 1.0 per cent K_2HPO_4.

Organisms from *L. japonicus* produce an acid fermentation of all of the sugars except maltose, raffinose, and dextrin; poor growth on starch agar without hydrolysis; fair growth on fructose, raffinose, and dextrin; moderate growth on arabinose, glucose, lactose, and sucrose; and abundant growth on maltose agars. They produce a moderate growth and acid fermentation of the glucosides amygdalin and salicin; a neutral reaction on aesculin agar (neutral red indicator); moderate growth and acid fermentation of the alcohols dulcitol, erythritol, and glycerol; poor growth and alkaline reaction on glycol medium; fair growth and alkaline reaction on sodium succinate agar; and very poor growth with a neutral or alkaline reaction on potassium oxalate and sodium urate media.

Organisms from *Lathyrus latifolius* produce an acid reaction on all the sugar media except raffinose and dextrin, with poor growth and partial hydrolysis of starch reported. They show fair growth on raffinose and dextrin; moderate growth on maltose, lactose, and sucrose; and abundant growth on glucose and fructose agars.

TABLE 2

Cross-inoculation results

HOST PLANT	TOTAL NUMBER OF ORGANISMS	ORIGINAL HOST	COWPEA	SOYBEAN	SWEET PEA	GARDEN PEA	SPRING VETCH	LUPIN	CLOVER	ALFALFA	GARDEN BEAN	*Genista tinctoria*	*Genista praecox*	*Baptisia tinctoria*	*Baptisia australis*	*Cassia nictitans*	*Cassia marylandica*	*Robinia pseudo-acacia*	*Cytisus hirsutus*	*Lathyrus maritimus*	*Lespedeza bicolor rosea*
Cowpea-soybean type:																					
Amphicarpa bracteata	20	20+*	8+	1± 2−	2−	1−	2−	2−	1−	2−	1−	1+		1+			2−	2−		1−	
Baptisia tinctoria	24	24+	9+ 1± 3−	4+ 9−											3+						
Cassia nictitans	27	27+	22+ 5±	4+ 2± 16−		8−		15−	14−	8−	9−	3+		2+			11−				
Crotalaria sagittalis	5	5+	5+	4−		2−		4−	4−	2−	2−	2+		2+							
Desmodium paniculatum	10	10+	2+ 8−	5+ 1± 6−																	
Dolichos Lablab	2	2+	2+	1+ 1−		2−		1−	1−	2−	1−	1+		1+		1+					

Genista tinctoria	9	9+	6+ 1−	1+ 6−									2+						2+		
Lespedeza hirta	3	3+	2+																		2+
Lespedeza frutescens	2	2+	2+																		2+
Totals	102	102+	58+ 6± 12−	15+ 4± 44−	2−	13−	2−	22−	20−	14−	13−	7+	2+	6+	3+	1+	13−	2−	2+	1−	4+
Pea type:																					
Lathyrus latifolius	3	3+			2+ 1±		3+														
Lathyrus japonicus	13	13+			8+ 2± 1−	1+ 1± 9−	1+ 5−		2−	2−	2−	1−		1−							
Totals	16	16+			10+ 3± 1−	1+ 1± 9−	4+ 5−		2−	2−	2−	1−		1−							

* The numbers refer to the number of strains of the organism which were tested, and the + and − to the resulting nodulation. Two or more pots, each containing three to six plants, were inoculated with each strain.

The *L. japonicus* organisms utilize leucine and, to a less extent, tyrosine as sole carbon and nitrogen sources, producing an alkaline reaction in each case, show an alkaline reaction on cystine medium, are inhibited by a solution containing 0.5 per cent alanine, and show decreased growth with 0.2 per cent. There is no increase in pH after growth in a solution containing a mixture of amino acids over that occurring when these are present singly. Growth on amino acid agar is increased by the addition of 0.008 per cent accessory growth factor, shows little or no further increase on the addition of 0.05 per cent KNO_3, but marked increase in growth and change of reaction from alkaline to strongly acid on the addition of 1.0 per cent mannitol.

All strains produced medium-sized, smooth, round to elongated nodules on the tap root or laterals near the tap root of the original host plant. Strains of the *L. japonicus* organism usually produced nodules on *L. odoratus*, rarely on *Pisum sativum* or *Vicia sativa*, and never on any other legume tested. Organisms from *L. latifolius* produced nodules on *Vicia villosa*, and irregularly on *L. odoratus*.

DISCUSSION

The foregoing characterization of group 1 agrees generally with the results of previous workers who found, in part, that the organisms from *Baptisia tinctoria*, *Genista tinctoria*, and certain species of Amphicarpa, Cassia, Desmodium, and Lespedeza were monotrichous, slow-growing rods, showing a thin, scant growth on ash agar, meager growth on potato, no liquefaction of gelatin, and no change on milk [Burrill and Hansen (1917), Löhnis and Hansen (1921), Shunk (1921), Müller and Stapp (1925)], and that the organism from a species of Crotalaria may pass through a cycle of short, motile rods, cocci, and banded rods [Gangulee (1926b)], whereas those from species of Desmodium are short rods showing very little branching or irregularities (10). Stapp (1923) reported a negative tyrosinase test on the one strain of *Genista tinctoria* organism which he studied, but nine strains of the organism were here found to produce no appreciable amount of tyrosinase. The alkaline (10 days) to neutral (2 weeks) fermentation of glucose and the neutral reaction on sucrose agar by a culture from a species of Lespedeza [Schönberg (1929)] are in agreement with the alkaline to neutral reactions resulting from growth of the Lespedeza cultures on these media in the present investigation.

The organisms from group 1 resemble those isolated from cowpea and soybean because of their monotrichous flagellation; swollen, vacuolated, unbranched bacteroids; slow growth of scant to moderate amount; inability to produce gelatinase or caseinase; and failure to grow well on potato [Burrill and Hansen (1917), Löhnis and Hansen (1921), Wright (1925a, b)]. In addition, they show marked similarity to both of these organisms in their ability to utilize the common monosaccharides, disaccharides, and trisaccharides, dextrin, certain glucosides, alcohols, and, to some extent, certain organic acid salts, producing, in most cases, an alkaline fermentation; in their more abundant growth and frequently acid reaction on a pentose sugar medium; and in

their failure to hydrolyze starch [Baldwin and Fred (1927), Wilson (1917), Walker and Brown (1930)]. The alkaline reaction produced by these cultures on litmus milk, usually accompanied by no other change, is also a characteristic of the cowpea and soybean organisms [Wright (1925a, b), Stevens (1925b)]. The cultures of group 1 resemble the cowpea organism and certain strains of the soybean organism in their failure to produce tyrosinase, although variations in this characteristic occur among soybean strains [(2), Stapp (1923)].

Further evidence of the relationship of the organisms of group 1 to the cowpea and soybean cultures was found in the cross-inoculation studies, in which certain strains from every host plant of this group formed nodules on cowpeas or soybeans, although irregularities in both of these crosses occurred.

Previous workers, on the basis of inoculation tests with one strain of the organism from *Baptisia tinctoria*, *Cassia nictitans*, and *Genista tinctoria*, included these legumes in the cowpea group [Burrill and Hansen (1917), Leonard (1923a), Walker (1928)] and also certain species of Crotalaria, Desmodium, and Lespedeza, as well as *Dolichos Lablab* (the latter having produced nodules when inoculated with the cowpea organism) [Gangulee (1926b), Richmond (1926a), Carroll (5), Burrill and Hansen (1917), Whiting and Hansen (1920), Löhnis and Leonard (1926), Pieters (1927)].

There is also some evidence in previous literature of the production of irregularities in nodulation of cowpeas by these organisms. For example, Koch and Butler (1918) reported negative results from inoculating cowpeas with *Lespedeza striata* cultures, since nodules were not formed every time the experiment was made, but positive results, though poor, from inoculating velvet bean, a member of the cowpea group. Cultures from *L. striata* and *Cassia chamaecrista* formed nodules on lima beans, which belong to the cowpea group, but in two cases results with the latter cross were negative the first time it was attempted [Whiting and Hansen (1920)]. Similarly, Burrill and Hansen (1917) found that *C. chamaecrista* organisms caused nodules on cowpeas but that in one case out of four a cowpea culture of proved nodule-forming ability failed to cause nodules on *C. chamaecrista*, and they also presented evidence, although they considered it inconclusive, that cultures from *Cassia nictitans* failed to produce nodules on *C. chamaecrista*, *C. medsgeri*, and *Vigna sinensis*.

Inoculation of soybeans by organisms from *Lespedeza striata* and *Desmodium tortuosum* [Leonard (1923a)] or by the organism from *Crotalaria juncea* [Gangulee (1926b)] has been reported as negative, but Leonard (1923a) stated that a strain isolated from *Cassia nictitans* formed nodules on both cowpea and soybean.

Bearing further on this question are the results of Leonard (1923a), Sears and Carroll (1927), Carroll (5), Walker and Brown (14), and others who have noted that organisms from cowpeas irregularly form nodules on soybeans and that the soybean organisms almost always produce nodules on cowpeas, thus suggesting a definite, though irregular relationship between these two cultures.

The present investigation on the cross-inoculation position of the legumes

of group 1 has brought to light so many irregularities that it is difficult to draw any clear-cut conclusions beyond the fact that the organisms from this group of legumes are related to one another and to both the cowpea and soybean organisms on the basis of their cultural characteristics and also of their cross-inoculation capacities. It has generally been considered that if an organism isolated from one legume will form nodules on another legume these two plants belong in the same inoculation group. Accordingly, *Crotalaria sagittalis* appears to belong to the cowpea group, since all strains produced nodules on cowpeas, and to belong in the same group with *Genista tinctoria* and *Baptisia tinctoria*, since it also formed nodules on these plants. Organisms from both *Genista tinctoria* and *Baptisia tinctoria* produced nodules on cowpeas; therefore, in spite of the fact that a few strains gave negative results on cowpeas in the tests made, their position in that group seems warranted, and a definite relationship to *Rhiz. japonicum* is indicated by their ability irregularly to form nodules on soybeans. *Amphicarpa bracteata*, although previously considered to belong to none of the known inoculation groups [Burrill and Hansen (1917)], *Dolichos Lablab*, *Lespedeza hirta*, and *L. frutescens* seem rightly to be members of the cowpea group, since all strains from these plants which were tested on cowpeas gave positive results and since the strains tested on *Baptisia tinctoria* and *Genista tinctoria* also produced nodules. Some positive and some negative results on soybeans with cultures from Amphicarpa and Dolichos are evidence of their irregular relation to *Rhiz. japonicum*. A strain of the Dolichos organism also formed nodules on *Cassia nictitans*, and cultures from the latter produced nodules on cowpeas, Genista, and Baptisia; hence, inclusion of *Cassia nictitans* in the same cowpea group seems justified. Irregularity in nodulation of soybeans is characteristic of the organism from Cassia, and, in addition, certain strains did not consistently form nodules on cowpeas. Their failure to form nodules on *Cassia marylandica* is supported by the observation of Leonard (1925b) that this species of Cassia bears no nodules. The organisms from *Desmodium paniculatum* showed irregularity in nodulation of both cowpeas and soybeans; hence the position of this legume in either group, as defined at present, is difficult to establish. The cultural reactions of the organism showed a relation to both cowpea and soybean organisms, as did the inoculation results, but the latter indicated a closer relation to *Rhiz. japonicum* than was noted with other members of group 1, because the percentage of positive results, the size and position of nodules, and the benefit to the host were greater on the soybeans.

The characterization previously drawn of group 2 is in agreement with that given by earlier workers of organisms from other species of Lathyrus, which they described as simple, peritrichous rods, often showing "X"- and "Y"-shaped, or large Indian-club, forms when old, and characterized by very fast, spreading growth, with abundant gum, on ash agar slants, a "good" growth on nitrogen-free maltose agar, and inability to produce tyrosinase [Schneider (1892), Beijerinck (1888), Burrill and Hansen (1917), Fred (1913), Shunk (1921), Harrison and Barlow (10), Stapp (1923)].

The organisms from group 2 resemble *Rhiz. leguminosarum* from Pisum and Vicia because of their peritrichous flagellation, branched bacteroids, abundant and rapid growth, inability to produce tyrosinase, capacity to form a serum zone and alkaline reaction on litmus milk and to utilize the common monosaccharides with the production of acid, certain disaccharides, trisaccharides, and alcohols with an acid or alkaline reaction, and certain glucosides, and, to some extent, organic acid salts with a usually alkaline reaction [Prucha (1915); Fred, Whiting, and Hastings (1926); Löhnis and Hansen (1919); Stevens (1925b)].

Cross-inoculation tests with cultures of group 2 confirmed their relationship with *Rhiz. leguminosarum*, but also brought out certain irregularities. *Lathyrus latifolius* has previously been placed in the pea inoculation group [Burrill and Hansen (1917)], and in these tests the *L. latifolius* organism formed nodules on both *L. odoratus* and *Vicia villosa*, although in the former case the nodules were small and few in number.

Other species of Lathyrus have been found to cross-inoculate within the genus and to form nodules on Pisum and Vicia [Simon (1914), Burrill and Hansen (1917), Carroll (5)], but *Lathyrus japonicus* has not previously been placed in any group. In the present investigation, the organisms from *L. japonicus* usually produced nodules on *L. odoratus*, but rarely on *Pisum sativum* or *Vicia sativa*. Since *L. odoratus* was placed in the pea group by Burrill and Hansen (1917), positive cross-inoculation with this plant would indicate that *L. japonicus* belongs to the same group. Variability among *L. japonicus* strains in producing nodules on pea and vetch suggest, as in the cowpea-soybean group, that the capacity to produce nodules on any legume other than the original host may not, under certain conditions, be a constant characteristic among all strains of a given organism, but rather a physiological strain characteristic, as suggested by Walker and Brown (14) in connection with the cowpea-soybean crosses. The irregularities in nodule production within the pea group, although not explained, are substantiated by evidence from previous reports of variability in cross-inoculation capacity among species of Vicia [Garman and Didlake (1914)], between Pisum and Vicia [Harrison and Barlow (10; 1907), Koch and Butler (1918)], and between Vicia, Lathyrus, and Pisum [Jardine (1924, 1926)], and of marked host specificity within the pea group [Helz, Baldwin, and Fred (1927)]. It is also noteworthy that *Vicia acutifolia* and *V. Floridana* have recently been placed in a new inoculation group (5).

The other physiological characteristics of these two groups of organisms supplement the cultural methods already known for separating them, and in a few cases may be of use in distinguishing the organisms from different hosts within a given inoculation group.

None of the organisms caused any visible color change after 2 weeks' incubation in neutral red glycerol ammonium phosphate solution, and since several common contaminants decreased the pH with resulting change of indicator

color from red to yellow, a lack of such change may be a check on the purity of at least some of the Rhizobia.

Sensitivity to 0.005 per cent dibrom-oxymercuri fluorescein in mineral agar separated group 2, which showed greatly impaired growth, from group 1, which grew nearly as abundantly as the controls. This concentration of dye also caused marked stimulation in growth of the Desmodium cultures, which exceeded that of the controls and was thus a distinguishing characteristic. Strains from Baptisia failed to grow, those from Genista grew very slightly with 0.5 per cent dye concentration and other members of the group showed a fair growth. In this, as in all other experiments where growth was judged by comparison of visible bacterial mass, increase in apparent growth may be either cell increase or increased gum production.

Complete inhibition of growth by K_2HPO_4 in concentration of 0.5 per cent or more, was the only result obtained from varying the concentrations of the salts used in standard nitrate mannitol agar which was consistent among all strains. An increase in growth over the control which was noted with most of the cultures on 0.1 per cent K_2HPO_4 agar has been previously reported by Truesdell (1917) with the alfalfa organisms. Separation of groups 1 and 2 might be effected by the use of 0.05 per cent $MgSO_4$ in the agar, since this caused a marked increase in the growth of most strains in group 1 and a slight decrease with the Lathyrus organisms.

On the sugar media, the organisms of group 1 usually resembled the cowpea and soybean cultures in growth and reaction, but a few exceptions must be noted. The organisms from Amphicarpa, Cassia, Desmodium, and *Lespedeza frutescens* generally produced an alkaline reaction in 2 weeks on arabinose agar, whereas the cowpea organisms produced acid in that period [Baldwin and Fred (1927)]. Most of the Cassia strains produced a less alkaline reaction on certain sugar media than did the cowpea organisms [Baldwin and Fred (1927)], and the cultures from Dolichos grew more rapidly than the other members of the group. These differences might be eliminated if a greater number of strains were studied. The cultures from *Lathyrus japonicus* and *L. latifolius* usually gave the same reactions on the sugar media as did *Rhiz. leguminosarum* [Baldwin and Fred (1927)], but on fructose, lactose, and sucrose agars more acid was formed by the beach pea than by the garden pea organisms, and on raffinose agar the reaction from the garden pea organism ranged from slightly alkaline to neutral, and that from the beach pea bacterium from moderately alkaline to moderately acid. *L. latifolius* strains gave the weakly acid fermentation of fructose and the alkaline fermentation of raffinose given by the garden pea organism, but resembled the strains from beach pea in producing slightly more acid on sucrose and considerably more acid on lactose than the garden pea cultures. They also produced slightly more acid from maltose than did those from *Pisum sativum* and considerably more than did the *L. japonicus* strains.

The cultures of group 1 gave an alkaline reaction on the glucoside media,

whereas the *L. japonicus* organisms produced an acid fermentation of salicin and amygdalin, thus separating the two groups and also distinguishing the latter from the pea organism, which showed a neutral reaction on these two media [Baldwin and Fred (1927)]. Apparent failure of the Lathyrus organisms to produce acid from aesculin may have been due to the use of neutral red in place of brom thymol blue indicator.

Growth of all the organisms on the media containing various alcohols was less abundant than with the glucosides except in the case of glycerol. The poor growth on dulcitol agar is interesting, since the isomeric hexahydroxy alcohol supports very good growth. The acid fermentation of glycerol by the Lathyrus organisms and the alkaline fermentation by the members of group 1 may separate these two groups.

Of the organic acids used as a source of carbon and energy, sodium urate and potassium oxalate supported practically no growth, the only indication of any metabolic activity being a slight increase in pH in some cases. The two groups reacted similarly on the organic acid media, and fair growth with an alkaline reaction on sodium succinate agar was characteristic of all but the Amphicarpa cultures, which displayed poor growth and a neutral reaction.

The growth of the Rhizobia on a mineral medium containing various amino acids show that these organisms, like certain others (3, 4, 13), are capable of utilizing particular amino acids for both their carbon and their nitrogen metabolism. Growth is usually accompanied by an increase in pH probably due to an accumulation of NH_3 resulting from deamination. Failure to show much increase in pH on tyrosine solution is in accordance with the results of Pohlman (1931c), who found that *Rhiz. meliloti* and *Rhiz. japonicum* utilized tyrosine slightly as a source of nitrogen, but without the production of ammonia, and hence may not, in all cases, have indicated failure to grow. Blanchetière (4) found, by analysis for total and ammoniacal nitrogen, that amino acids which are not attacked singly by *Bact. fluorescens* are attacked in a mixture. The increase in pH produced in a solution containing a mixture of four amino acids, totaling 0.5 per cent concentration, was far greater than occurred in solutions containing any of the amino acids singly, in the case of all but the Lespedeza, Dolichos, and Lathyrus cultures, indicating a similar situation within the genus Rhizobium. Whether this increased alkalinity is due to increased growth previously limited by the absence of a specific amino acid essential either for growth or for the formation of an enzyme needed to break down other amino acids, or whether an unknown factor is responsible is a matter for conjecture at present. These results are given in table 3.

The addition of 0.008 per cent growth factor to the amino acid agar produced a marked increase in the visible bacterial mass. Since this amount of the factor is equivalent to less than 0.006 per cent carbohydrate, it seems probable that the increase resulted not from the small amount of carbon added, but from a definite stimulatory effect. The failure to show much further increase when 0.05 per cent KNO_3 was added indicated that these amino acids

serve nearly as adequately for nitrogen metabolism as does potassium nitrate. One per cent mannitol, however, increased the bacterial mass on leucine and tyrosine media to an amount equal to or exceeding that of the control. The amino acid thus appears to be a less available source of carbon than the mannitol, but the improved conditions for growth brought about by the

TABLE 3

Reactions produced by cultures on a mineral solution containing amino acids of 0.5 per cent total concentration

SOURCE OF CULTURE	ALANINE*	CYSTINE	LEUCINE	TYROSINE	ALANINE, LEUCINE, CYSTINE, TYROSINE
	pH	*pH*	*pH*	*pH*	*pH*
Amphicarpa bracteata	7.0(2)†	7.0(2)	7.0(3)	6.8(3)	7.6(2)
	6.8(1)	7.2(1)			7.4(1)
Baptisia tinctoria	7.0(2)	6.8(2)	7.2(3)	6.8(2)	7.6(2)
	7.2(1)	7.0(1)		6.6(1)	7.4(1)
Cassia nictitans	7.0(2)	6.6(2)	7.2(2)	6.8(3)	7.6(2)
	7.2(1)	7.0(1)	7.0(1)		7.4(1)
Crotalaria sagittalis	7.0(2)	6.8(2)	7.0(3)	6.6(1)	7.6(2)
	7.2(1)	6.6(1)		6.7(1)	7.4(1)
				6.8(1)	
Desmodium paniculatum	7.0(2)	7.0(2)	7.0(3)	6.8(3)	7.6(2)
	6.8(1)	6.8(1)			7.4(1)
Dolichos Lablab	7.4(1)	6.0(2)	6.6(2)	6.2(1)	6.8(1)
	7.0(1)			7.0(1)	7.2(1)
Genista tinctoria	7.2(2)	6.8(3)	7.0(2)	6.8(2)	7.6(3)
	7.0(1)		7.2(1)	6.7(1)	
Lespedeza frutescens	7.0(2)	7.0(1)	7.0(2)	6.8(1)	6.8(2)
		7.2(1)		7.0(1)	
Lathyrus japonicus	7.0(3)	7.4(3)	7.0(3)	7.2(2)	6.9(3)
				7.0(1)	

* 0.2 per cent.

† Numbers in parentheses indicate the number of strains producing the given reaction.

production of acid from mannitol simultaneously with the production of ammonia from the amino acid must not be disregarded. The average results from twenty-five representative strains from each group are presented in figure 1, based on four arbitrary units of visible bacterial mass.

In a solution containing 0.5 per cent alanine, growth of all cultures was inhibited; in 0.2 per cent concentration growth was not so abundant as the con-

trol, even when 1.0 per cent mannitol was present. This toxicity of alanine may be comparable to the results of Pohlman (1931c), who found that 0.05 to 0.1 per cent glycocoll was toxic to certain strains of *Rhiz. japonicum.*

The very alkaline reaction produced by the *L. japonicus* strains on cystine solution appears to separate these organisms from those of group 1, and the change from scant growth with neutral or alkaline reaction on leucine or tyrosine agars, with or without the growth factor or nitrate, to abundant growth with strongly acid fermentation when mannitol is present is also char-

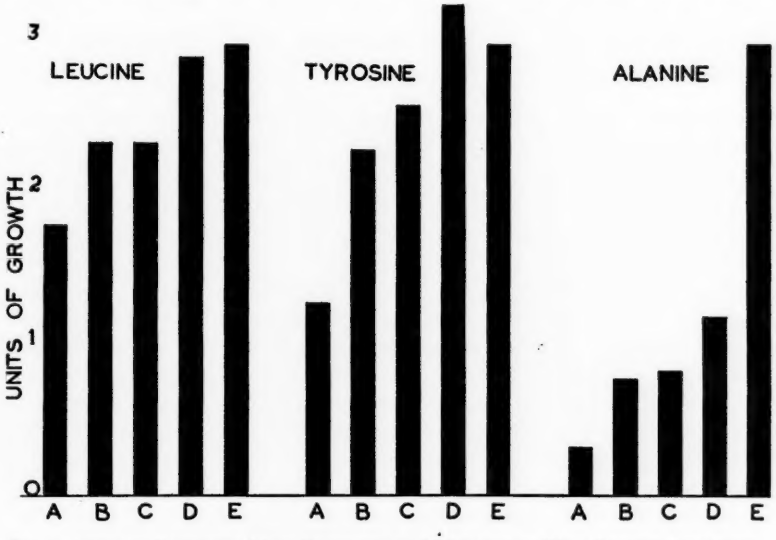

FIG. 1. AVERAGE BACTERIAL MASS PRODUCED BY 25 STRAINS OF WILD LEGUME ORGANISMS ON A MINERAL AGAR

The mineral agar contained:

A. 0.5 per cent amino acid (0.2 per cent in the case of alanine).

B. 0.5 per cent amino acid plus 0.008 per cent growth factor.

C. 0.5 per cent amino acid plus 0.008 per cent growth factor plus 0.05 per cent potassium nitrate.

D. 0.5 per cent amino acid plus 0.008 per cent growth factor plus 1.0 per cent mannitol.

E. Control. Standard nitrate mannitol agar.

acteristic of the bacteria from *L. japonicus.* The strong production of acid on cystine solution and the slightly acid reaction on leucine solution might separate the Dolichos strains from other members of group 1. The Baptisia cultures showed some growth and an alkaline reaction on alanine agar without the growth factor, thus separating them, with two exceptions, from the other members of group 1 which were unable to grow on this medium.

The results of these cultural studies in most cases showed considerable variation among strains of an organism from any one host. This was particu-

larly true in both the rate and amount of growth of group 1 and in the dye and salt sensitivity of the organisms studied. On the carbohydrate media, variations in reaction between strains from the same host occurred occasionally, even between isolations from the same nodule. Although Dunham and Baldwin (1931), basing their distinction between strains on the grounds of inoculation effect, agglutination reaction, and certain cultural characteristics, concluded that in no case were there two strains in a single nodule, the differences in the nature of the colonies formed by different organisms which they noted may be comparable to the slight strain variations found here in fermentation reaction.

SUMMARY

Organisms causing nodules on ten wild legumes and on *Dolichos Lablab* were studied from the standpoint of their morphological, cultural, and cross-inoculation characteristics.

Cultural characterization of these organisms has been extended to include their fermentation of numerous carbohydrates, their reactions on amino acid media, and their sensitivity to salts and dye.

These organisms appear to fall into two groups.

Group 1 includes the organisms causing nodules on *Amphicarpa bracteata*, *Baptisia tinctoria*, *Cassia nictitans*, *Crotalaria sagittalis*, *Desmodium paniculatum*, *Dolichos Lablab*, *Genista tinctoria*, *Lespedeza hirta*, and *Lespedeza frutescens*.

The morphological and cultural characteristics of the members of group 1 are essentially the same as those of the organism from cowpea and the organism (*Rhizobium japonicum*) from soybean.

On the basis of inoculation experiments, the organisms of group 1 are further characterized by their ability to form nodules, in so far as tested, on any other legume of the group, and by the ability of nearly all strains to form nodules on cowpeas, and of certain strains on soybeans. Cultures from *Desmodium paniculatum* appear to form nodules more readily, and the nodules are of a more beneficial type, on the latter.

Further evidence of the relationship of the organisms of group 1 to the cowpea and soybean organisms is the occurrence of cross-inoculation irregularities among strains of the latter. This suggests that the capacity to form nodules on any but the original host plant may be a physiological characteristic not found under certain conditions in all strains of these Rhizobia.

The strains of group 1, therefore, appear to be closely related to one another and also to the cowpea and soybean organisms both in cultural and cross-inoculation characteristics. Variations in their cross-inoculation capacities, however, prevent the giving of a species name to these organisms or the definite placing of their host plants in either the cowpea or soybean inoculation group, if the present bases for determining species and inoculation groups be observed.

Group 2 includes the organisms causing nodules on *Lathyrus latifolius* and *Lathyrus japonicus*.

The morphological and cultural characteristics of the organisms of group 2 are essentially the same as those of *Rhizobium leguminosarum* from Pisum, Vicia, and certain species of Lathyrus.

The organism from *L. latifolius* is further characterized by its ability to form nodules on Vicia and on other species of Lathyrus and hence should be included in the pea group as previously suggested.

The organism causing nodules on *Lathyrus japonicus* is characterized by its ability to form nodules on other species of Lathyrus and by its irregularity in forming nodules on Pisum and Vicia. This irregularity, similar to that found among cultures of group 1, prevents the placing of *Lathyrus japonicus* without reservations in the pea group, or the conclusion that under the present species rules, the organism is *Rhizobium leguminosarum*.

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THE NITRIFICATION PROCESS AND PLANT NUTRITION[1]

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Nitrate is the most abundant anion, except bicarbonate, resulting from biological processes in good soils. Likewise, if it is assumed that the plant takes its nitrogen in the nitrate form, nitrate is needed in larger amounts by plants than is any other anion. Nitric acid is strong and has a vigorous solvent action upon soil minerals.

What restricted nitrification, what processes in the soil take its place? Some plants apparently succeed where there is little nitrification. What is the significance of the nitrification process in the nutrition and growth of higher plants? The following considerations attempt to answer these questions.

THE SOLVENT ACTION OF NITRIFICATION

The significance of nitrate as a carrier of cations cannot be appreciated from the quantity found at any given moment. The nitrate level in the soil at any time under crops may be low even though the nitrate has served in liberating from the soil and carrying into the plant a large portion of the cations needed for growth.

The large amounts of nitrate that may be produced and the solvent effect of nitrification are shown by calculations from data on an uncropped soil studied by Hibbard (14). The data are presented in parts per million by Hibbard, but the writer has converted them to milliequivalents per 10,000 gm. soil (table 1).

The data in table 1 indicate that the nitrate in the lower concentrations accounts for only a small part of the soluble cations but, as the nitrate increases, soluble cations increase markedly, and the nitrate accounts for a larger portion of their total. In the low concentrations of nitrate the sulfate is of about equal importance. With increased biological action the nitrate becomes increasingly important as a solvent, compared with sulfate. Perhaps in the lower concentrations of nitrate in non-acid soils, as suggested by Burd (5), most of the cations are held in soluble form as bicarbonates. When nitrate increases, the solvent effect of carbonic acid becomes correspondingly less significant.

With nitrate present in large amounts, cations must be in solution in relatively large amounts, though the converse is not necessarily true. Other anions can, of course, hold cations in solution.

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RELATION OF SOIL REACTION TO THE SOLVENT EFFECT OF NITRIFICATION

The problems of plant nutrition are not identical in acid and alkaline soils. Carbonic acid partially neutralizes black alkali by forming a bicarbonate.

Data by Conrad (8) indicate that carbonic acid has little solvent effect upon soils more acid than pH 6.0. The dissociation constant for carbonic acid (6) likewise indicates that an acidifying and therefore solvent effect cannot be appreciable until the soil is about neutral or somewhat alkaline in reaction. McGeorge and Breazeale (21) showed that an alkaline calcareous soil of pH 8.4 could be reduced in a water suspension to pH 6.4 by carbonic acid.

Saline soils carry a relative abundance of salts in solution, largely chlorides and sulfates. Acid soils are the result of excessive leaching and removal of solutes. The more easily dissolved nutrients have long since disappeared. Solutes useful to plants, as they are liberated from day to day, must be brought into available form largely by biological processes that produce solvents capable of attacking the somewhat inert mineral soil. Under such conditions strong acids, such as nitric, must be the principal active solvents.

TABLE 1

Water extract of Yolo silty clay loam soil untreated (1–5), showing important nutrients

M.e. per 10,000 gm. soil

TIME OF INCUBATION	NO_3	PO_4	SO_4	SUM ANIONS	K	Ca	Mg	SUM CATIONS	CATIONS ACCOUNTED FOR BY NO_3
days									*per cent*
0	4.2	1.6	4.5	10.3	7.2	19.1	14.9	41.2	10
11	9.0	1.4	6.5	16.9	8.8	24.9	16.6	50.3	18
40	16.4	2.1	4.0	22.5	5.8	36.7	16.6	59.1	28
90	18.6	1.7	3.9	24.2	9.3	34.7	19.3	63.3	29
160	23.7	1.7	5.8	31.2	11.4	23.0	20.4	54.8	43
254	46.1	1.5	13.0	60.6	10.8	20.9	34.9	66.6	69
395	51.1	1.6	10.0	62.7	12.5	25.5	29.6	67.6	76

When active nitrification starts in the spring, soil acidity increases. The increase may amount to as much as 1 pH during the season but usually it is less (13, 24, 25). Later in the season, vigorous growth of plants depletes the soil of its nitrates, and the acidity is reduced. After harvest, another period of active nitrification again increases acidity. Changes in soil reaction follow closely upon changes in the accumulation of nitrates.

The solvent effect of nitrification is observed in the removal of bases in the form of nitrates leached from the soil during the winter (15). The leaching losses through a 4-year period at the Virginia Agricultural Experiment Station are summarized in table 2. Soils were treated in different ways, and the calcium, magnesium, potassium, sulfate, and nitrate that were removed by drainage waters were estimated. Soil was exposed in tanks, and the drainage water was collected for analysis. For convenience of quantitative comparison, the

losses of the various nutrient ions have been converted to hydrogen equivalents per acre. The data indicate that the nitrate loss is sufficient to account for two-thirds to three-fourths the cations removed. The pH of the soil is not given, but the leachings were nearly neutral.

TABLE 2

Summary of 4 years' leaching losses of Ca, Mg, K, SO_4, and NO_3 converted to hydrogen equivalents per acre (15)

SOIL TREATMENT	GREEN RYE MULCH	GREEN RYE TURNED	MATURE RYE MULCH	MATURE RYE TURNED	CONTROL
Cation loss (Ca + Mg + K)	34.10	34.65	26.88	24.42	21.48
Anion loss (SO_4 + NO_3)	29.61	28.71	21.59	19.78	17.29
NO_3 loss	25.69	25.43	17.09	16.56	14.13
Cation loss accounted for by NO_3 ..... *per cent*	75.3	73.4	63.6	67.7	65.7

TABLE 3

Important nutrients—nitrate, sulfate, calcium, and potassium—water soluble (1:5) in the soil

M.e. per 10,000 gm. soil

SOIL TREATMENT	ANIONS†		SUM ANIONS	CATIONS		SUM CATIONS	DIFFERENCE CATIONS AND ANIONS	Ca + K ACCOUNTED FOR BY NO_3
	NO_3	SO_4	NO_3 + SO_4	Ca	K	Ca + K	Excess Ca + K over NO_3 + SO_4	
								per cent
Meyer clay adobe:								
Check	17.38	7.04	24.42	26.04	4.95	30.99	+6.57	56
*Alfalfa 5 tons	26.19	5.49	31.68	42.00	7.18	49.18	+17.56	53
Manure 5 tons, dry weight	23.33	7.99	31.32	30.16	7.27	37.43	+6.11	62
Aiken silty clay loam:								
Check	14.67	2.14	16.81	10.10	2.70	12.80	−4.01	115
Alfalfa 5 tons	31.67	3.12	34.79	20.33	6.16	26.49	−8.30	119
Manure 5 tons, dry weight	22.38	4.03	26.41	13.50	7.69	21.19	−4.22	105

* Rates of treatment are for 2,000,000 pounds of soil. Treatments were made on potted soils in the greenhouse.

† The term "ion," in this paper is used in a general way, without any distinction between the ionized or the non-ionized form.

The data in table 3 indicate how the reaction of the soil and the reserve supply of mineral nutrients may affect the solvent action of biological processes. The soils were treated with two nitrifiable materials, alfalfa and stable manure.

The Meyer soil is nearly neutral in reaction and well supplied with the important nutrient cations. The exchangeable calcium is 0.630 per cent, and the exchangeable potassium is 0.091 per cent. There is more water-soluble calcium

and potassium than is equivalent to the nitrate and sulfate, indicating an active solvent effect of some other acid, hydrochloric or carbonic, probably the latter (5).

The Aiken soil is acid (pH 5.6) and base deficient. The exchangeable calcium is 0.120 per cent, and the exchangeable potassium, 0.038 per cent. To bring bases into solution in this soil requires the vigorous action of strong acids, nitric and sulfuric.

The result is that in the Meyer soil there is an excess of calcium and potassium over nitrate and sulfate and that in the Aiken soil there is a deficiency of calcium and potassium compared to nitrate and sulfate. Thus the reaction of the soil and the supply of cations in the soil complex determine how effective is the solvent action of the acids of varying strengths.

Nitrate in the acid soil is sufficient to account for more than both the calcium and potassium that are in soluble form. In the neutral soil, which contains approximately the same amount of nitrate and sulfate but much more calcium and potassium, the nitrate is equivalent to about half the calcium and potassium in solution, and other solvents are of greater significance.

THE COMPOSITION OF PLANTS AS INDICATIVE OF THEIR NUTRITION

Plants absorb from the soil and its solution anions which include NO_3^-, $SO_4^=$, Cl^-, $PO_4^=$, $SiO_3^=$, HCO_3^-, and possibly others. The cations are Ca^{++}, Mg^{++}, K^+, Na^+, Fe^{+++}, and others. The form in which absorption occurs is uncertain.

Table 4 shows four each of the more common anions and cations and their quantitative importance for a number of crops. The data are calculated from Forbes' analyses (12).

The table shows nitrogen in an amount more than equivalent to the sum of the four important cations in most cases. The nitrogen is generally considerably more than equivalent to the other three important anions. Omission of the silicate anion, which was not reported in the analyses, does not affect the main purpose of the discussion.

The presence of nutrient ions in the plant indicates nothing of how they may have been absorbed. In priority of liberation in the soil, greater importance attaches to the anions since they are, for the most part, liberated from organic matter by biological processes. Liberation of anions indirectly controls the liberation of cations.

Plants of high nitrogen content should be high in cations, if nitrate is an important carrier of cations. Parker and Truog (22) point out the relation between high nitrogen and high calcium, though they were studying the relationship from another point of view. A rather extensive study would be required to establish this, at present, assumed relationship.

A study of the table (an incomplete list) indicates that plants contain more anions than cations (nitrogen being considered as an anion), though there are not sufficient data for arriving at the absolute sum of either. The plant, of

course, maintains a balance of ions. The balance may be adjusted by conversion of nitrate to ammonia and by the synthesis of organic ions. If carbonic acid is included as a solvent and carrier of nutrients in soils of favorable reaction, a balance of anion and cation in plant intake is facilitated. Under some conditions, the hydrogen of the carbonic acid may correct a deficiency of cations.

Possibly nutrients can be absorbed as acids, nitric acid (1, 7) for example. Breazeale and McGeorge state that phosphate is best absorbed as the H_2PO_4 anion (4). If some of the nitrogen is absorbed as ammonia, a cation is furnished to balance anions.

But there is another helpful explanation providing for adequate absorption and nutrition of the plant without upsetting the balance of anion and cation if

TABLE 4

Anions and cations of common crops

M.e. per 100 gm.

CROP	ANIONS				SUM ANIONS	SUM OF S, Cl, P	N PER CENT OF TOTAL ANIONS	CATIONS				SUM CATIONS	CATIONS ACCOUNTED FOR BY N AS AN ANION
	N	S	Cl	P				K	Na	Ca	Mg		
													per cent
Alfalfa hay	165.0	17.5	4.2	21.3	208.0	43.0	79	19.7	19.5	51.5	30.3	121.0	136
Bluegrass hay	104.3	19.4	6.2	21.3	151.2	46.9	69	32.9	5.8	15.5	18.0	72.2	144
Clover hay	148.6	11.3	6.8	16.5	173.2	24.6	86	43.4	2.6	57.0	22.1	125.1	119
Millet hay	68.6	9.4	3.4	16.5	97.9	29.3	70	32.4	3.9	15.5	20.5	72.3	95
Cowpea hay	254.3	20.0	4.2	24.3	305.8	51.5	83	19.9	28.2	90.5	80.4	219.0	116
Timothy hay	60.7	9.4	5.1	10.7	85.9	25.2	71	14.3	13.9	9.0	8.2	45.4	137
Soybean hay	195.7	14.4	2.3	20.4	232.8	37.1	84	40.6	5.8	61.5	50.8	198.7	98
Wheat straw	20.0	9.4	5.6	3.9	38.9	18.9	51	20.4	9.5	10.5	4.9	45.3	44
Wheat grain	117.9	12.5	2.1	35.9	168.4	50.5	70	13.3	1.2	2.5	10.7	27.7	426
Corn stover	62.9	10.6	8.2	9.7	91.4	28.5	69	43.9	2.6	23.5	7.4	77.4	81
Corn grain	99.3	9.4	1.7	25.2	135.6	36.3	73	8.7	0.9	0.5	9.0	19.1	520
Cabbage	13.6	3.8	0.6	1.9	19.9	6.3	68	4.3	0.0	2.0	1.6	7.9	172

nitrogen is absorbed as the anion. Almost immediately in the plant, the nitrogen is changed to the cationic form of ammonia (11) and combined with fatty acids to form amino acids. The cation with which the nitrogen was associated in entering the plant is thereby released to unite with some other anion, probably the bicarbonate, a fatty acid, or a by-product of plant metabolism. Parker and Truog (22) believe that an important function of calcium is to neutralize acid by-products of metabolism, which might otherwise prove toxic to the plant.

When any solute, potassium nitrate, for example, enters the plant, the change of the nitrate anion to the cationic form of ammonia liberates the potassium. Some of the potassium or other cations so liberated may combine with carbonic acid (produced abundantly by cell respiration) to form a bicarbonate. The

cation thus combined may diffuse from the plant cell into the soil to serve again as a base for nitrification. The same cation may serve repeatedly to carry anions, and particularly the nitrate anion, into the plant. This provides for maintaining a balance between cations and anions though the latter are used in larger quantities for growth. Though absolute proof may be questioned, migration of nutrients from the plant into the soil has often been assumed.

Thom and Humfeld present data (26) which indicate that the soil immediately adjacent to plant roots is kept nearer neutrality than the soil farther way. The authors do not offer an explanation for the effect. If bases are capable of passing from plant roots into the soil, that would account for the reduced acidity in acid soils. The excretion of carbon dioxide by the roots and microorganisms would account for the reduced alkalinity in alkaline soils.

The foregoing postulation may account for the mobilization of nutrients for plants absorbing large quantities of nitrate. Is the situation different for legumes? Legumes usually absorb and assimilate large amounts of bases, especially calcium and potassium. The legume gets much of its nitrogen from the air through the organisms in its roots. This might result in a reduced absorption of nitrate from the soil and give rise to the problem of providing a solvent and carrier to mobilize the needed cations. But legumes absorb nitrate from the soil to the extent that it is available, and fix nitrogen only to meet additional needs.

In addition to the nitrogen supplied symbiotically, which probably averages nearly half the total nitrogen obtained, enough more would be needed from the soil in the form of nitrate to account for most of the cation needs. In addition, many legumes thrive only on soils well supplied with bases and nearly neutral or somewhat alkaline in reaction, a condition in which carbonic acid is more effective as a solvent. Plants can no doubt absorb bicarbonates.

ABSORPTION OF NUTRIENTS BY PLANTS IN ACID SOILS

As acidity and base deficiency in soils increase, the nutrition of most farm crops becomes correspondingly poor. In very acid soils nitrification may be greatly reduced and sometimes nearly absent. If nitric acid is an important nutrient and an effective solvent and carrier of cations, what can compensate for its lack when high acidity develops?

Ammonia, as shown by Allison (1, 2) and others (27), can be utilized by many plants but is poorly absorbed from acid solutions (27). Nitrate is more readily absorbed from an acid medium.

Ammonia does not dissolve mineral nutrients, and neither does it serve as a carrier of cations. In soils where more of the nitrogen is liberated in the form of ammonia, plants appear at a disadvantage in obtaining nutrients. Plants native to acid soils are low in cation content, as is indicated by a comparison of the mineral composition of conifers and hardwoods. The conifer growing in acid soil environment has adjusted itself to low mineral needs. The same relationship holds for pasture grasses grown on good and poor soils. As the soil

becomes acid and low in nitrifiable organic matter the better grasses give way to those of poor quality, low in both minerals and protein (9).

Joffe (18) presents ash analyses of litter (old leaves) from various sources for both conifers and hardwoods. After averaging several analyses he states: "The average ash content of conifers is 2.54 per cent, with a minimum of 1.29 per cent and a maximum of 3.31 per cent, whereas the average from the hardwoods is 6.63 per cent with a minimum of 3.85 per cent and a maximum of 11.74 per cent." The hardwood leaves average 2.6 times as high in ash as the conifers.

Plants probably are not entirely dependent upon solutes in the soil for nutrition. Jenny (17) has shown that the soybean is capable of obtaining calcium directly from the exchange complex of the soil colloids. Hydrogen was excreted by the plant root and exchanged for the calcium. Jenny found that the plant was at a disadvantage when compelled to take calcium from this source, after the calcium concentration in the colloid was reduced to a low level.

Nature makes some provision for the nutrition of plants which are native to acid, base-deficient, ammonia-producing soils. There is the possibility of a symbiotic relation between certain fungi and the host plant. The fungi, both endophytic and ectophytic, grow on the roots of the host, and the fungi-infected root, designated a mycorrhiza, is the absorbing organ of the plant. The mycorrhiza are abundant on acid plants, particularly conifers, heaths, and orchids. The natural habitat of these plants is in acid humus, or soils rich in acid humus, where little nitrate is produced.

The fungi have been found on the roots of many other plants, but on plants which grow in good nitrate producing soils only a pseudo-mycorrhizal development occurs. Jones (19) states that he found mycorrhizal roots on nearly all the common legumes wherever grown but that with favorable conditions rapidly growing roots soon outgrow the fungus. The fungus is present merely incidentally rather than as serving an important function.

That mycorrhiza are helpful to plants growing in acid soils has been both advocated and disputed by several workers (23). There are several points that are indicative of the helpfulness of the mycorrhiza. The young and actively absorbing roots develop the mycorrhiza. In some nursery soils forest trees fail because of lack of the proper fungi for the mycorrhiza. When the fungi are introduced and the mycorrhiza develop, the trees thrive (23).

Rayner (23) states that, "Conifers and, in all probability, other trees, can utilize ammonium compounds, and possibly to some slight extent, more complex organic compounds of nitrogen, but the last named are much more readily used by the root fungi. Hence, on acid humus soils, in which such compounds constitute the chief source of nitrogenous nutrient, plants provided with mycorrhiza are extraordinarily well equipped for competition with soil organisms." The organisms bringing about decomposition have first chance at the rather limited supply of available nitrogen of raw acid humus.

Rayner in the course of his discussion refers to "Stahl's theory of nutrition," as related to mycorrhiza. The mycorrhiza are a very efficient mechanism for absorbing soluble salts from the soil. Higher plants with abundant root systems and many root hairs are efficient absorbers. In mycorrhiza plants the fungus largely replaces the root hair as an absorbing organ. Rayner (23, p. 56) defines Stahl's theory of nutrition: "Namely, that the demand for available mineral salts in certain soils exceeds the supply, the resulting intensity of competition being the primary cause of a symbiotic relation in mycorrhiza." Thus the mycorrhiza are helpful to the plant not only in obtaining nitrogen, but in obtaining minerals also.

McArdle (20), in discussing the relation of mycorrhiza to conifers, states that the fungus may enable the trees to absorb water better, and may aid also in the absorption of mineral nutrients. Most of the mycorrhiza are found in the upper layers of soil where the tree gets its nutrients. He does not find any indication of injurious effects caused by the fungus of the mycorrhiza.

From the standpoint of energy economy, the absorption and utilization of ammonia should prove advantageous to plants. Nitrate is reduced to ammonia in the plant as the first step in protein synthesis. Results do not indicate an advantage, however, but rather a disadvantage. Lack of nitrate eliminates some species in a competitive existence. Others have become accommodated to an ammonia nutrition through symbiotic relation with fungi. But even these plants are probably better nourished in an environment where some nitrification occurs. In very acid soils there is often some nitrification.

Rayner again says (23, p. 216): "It is well to recall the fact that in the plant world, the severity of the struggle for existence not uncommonly centers about the competition for suitable compounds of nitrogen. . . .Even the rôle of vascular plants in general as 'nitrate organisms' may be so regarded, forming, as it does, an indispensable link in that remarkable cycle of chemical changes by which the 'circulation of nitrogen' is secured and maintained in nature."

GENERAL SIGNIFICANCE OF NITRIFICATION IN SOILS

There is good evidence that nitrification is of more than passive significance in plant nutrition. Nitrates are absorbed and renewed in the soil many times throughout the growing season. No other strong acid (in the form of its salt) is so abundant in any but saline or alkali soils. No other anion (if absorption of nitrogen in the anion form is assumed) is needed in such large amounts for plant growth.

With nitrification eliminated there are no adequate means for dissolving a sufficient quantity of the cations of the mineral soil (1) and for getting sufficient of either nitrogen or the cations into the plant (2). The mycorrhizal arrangement, while helpful to certain plants, can hardly be considered adequate. The mineral nutrition of crop plants on very acid soils is not satisfactory.

Building up the nitrifying power of poor sour soil through the addition of

high nitrogen organic matter and the use of lime to neutralize acids is favorable to the nutrition of common plants. Observation indicates that as soils become poor and base deficient, the renewal of organic matter which may support nitrification becomes increasingly important. Legumes may succeed with little organic matter if there is sufficient basic material to maintain the soil near neutrality.

Humid subsoils, usually somewhat acid, low in organic matter, and possessing little biological activity are characteristically raw, or refuse to give up mineral nutrients even to legumes which are presumably able to obtain sufficient nitrogen from the air. A bad physical condition is often assigned as the cause for the rawness. Perhaps it is not the only cause. Incorporation of organic matter and the stimulation of the nitrification process entirely change the subsoil character. Mineral nutrients are liberated and plant growth is supported. The subsoil of arid areas, by contrast, are usually not raw to legumes. These subsoils are alkaline in reaction and carry a good supply of easily soluble minerals. Carbonic acid given off by the roots of the plant is capable of bringing into solution and carrying into the plant some of the minerals so easily accessible.

Jenny (16) has correlated crop yields and land values on a broad basis with the nitrogen and organic content of soils. Although his data do not treat this phase, crop yields undoubtedly would correlate even more closely with the nitrifying power and the production of nitrates in the soil (28). Whenever conditions are favorable for complete mineralization of the organic matter, which means abundance of nitrate in soils that are rich in organic matter, there is also an abundance of available mineral nutrients important to plant growth. Among essential soil processes, therefore, nitrification stands high in its importance to the nutrition and growth of higher plants.

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SULFUR REQUIREMENTS OF AZOTOBACTER CHROOCOCCUM

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All living organisms, including bacteria, require sulfur for normal growth and metabolism. Some forms of life require it in the organic form, whereas others require it in the inorganic form. To which class does *Azotobacter chroococcum* belong? Some soils are extremely low in sulfur; hence, it may occasionally be the limiting factor in crop production (5). If sulfates are added to some soils low in sulfur, nitrogen fixation is increased (4), and when small quantities of such a soil are incubated in a liquid medium a profuse chocolate-brown membrane appears. The growth is often scant in quantity and of a light color when sulfates are deficient (1). The increased bacterial activity occurring when sulfur and sulfur compounds are added to soil may be due to sulfur acting as a nutrient or to the liberation from the soil of other essential nutrients, for it is rather generally known that sulfates are powerful soil stimulants (3). The work reported in this paper deals with the nitrogen fixed in pure cultures of *Azotobacter chroococcum* grown in a basic liquid medium to which colloidal sulfur and various sulfur compounds were added in varying concentrations. This rules out the indirect effect which may occur in soils and answers the question: What forms of sulfur are used by *Azotobacter chroococcum*?

METHODS

A strain of *Azotobacter chroococcum* isolated from the College Farm soil was used. It was cultured on a synthetic medium (2) and examined periodically to insure purity. The basic medium to which the colloidal sulfur and sulfur-carrying compounds were added had the following composition:

KH_2PO_4	0.02 per cent
$MgCl_2 \cdot 6H_2O$	0.02 per cent
NaCl	0.02 per cent
$CaCl_2 \cdot H_2O$	0.01 per cent
$FeCl_3$	50 p.p.m. iron
NaI	40 p.p.m. iodine
$MnCO_3$	40 p.p.m. manganese
$CaCO_3$	1.0 per cent
Mannitol	1.5 per cent
Distilled water	1,000 cc.

[1] Chemist and bacteriologist, and graduate assistant, respectively.

[2] Contribution from department of chemistry and bacteriology. Publication authorized by director, September 3, 1935.

Baker's chemicals of highest purity were used. No attempt was made to free them from sulfur impurities; hence, the basic medium always carried a minute constant of sulfur. To this basic medium were added, both separately and in varying quantities, mustard oil, sodium thiocyanate, sodium thiocarbonate, sodium sulfocarbolate, sodium ethyl sulfate, colloidal sulfur, sulfur chloride, sulfuryl chloride, sulfur iodide, sulfonmethane, sodium sulfanilate, sodium sulfide, sodium sulfite, cysteine, and ethyl mercaptan. One hundred cubic centimeter portions of the different media were distributed in 500-cc. Erlenmeyer flasks and sterilized in an autoclave. One-half of the flasks were inoculated with 1 cc. of a suspension of *Azotobacter chroococcum*; the other half were

TABLE 1

Nitrogen fixed in the presence of varying forms and quantities of sulfur

	NITROGEN FIXED IN MEDIA CONTAINING				
	No S	5 p.p.m. S	10 p.p.m. S	50 p.p.m. S	100 p.p.m. S
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Mustard oil (added after autoclaving)	1.2	1.4	1.3	0.9	0.6
Mustard oil (added before autoclaving)	1.2	1.3	1.4	1.4	0.5
Sodium thiocyanate	1.2	1.3	1.2	1.4	1.3
Sodium thiocarbonate	1.2	4.1	4.3	4.4	4.4
Sodium sulfocarbolate	1.2	1.3	1.2	1.2	1.4
Sodium ethylsulfate	1.2	4.1	3.9	4.4	4.5
Colloidal sulfur	1.2	3.8	3.0	4.0	4.0
Sulfur chloride	1.2	4.4	4.9	3.9	4.2
Sulfuryl chloride	1.2	5.2	5.0	5.0	3.4
Sulfur iodide	1.2	.....	0.8	0.5	.....
Sulfonmethane	1.2	1.3	1.3	1.4	1.4
Sodium sulfanilate	1.2	1.4	1.2	0.7	0.1
Sodium sulfide	1.2	4.6	4.6	4.3	4.6
Sodium sulfite	1.2	4.0	4.2	4.1	4.3
Cysteine hydrogen chloride	1.2	4.1	3.8	3.9	3.2
Ethyl mercaptan (added after autoclaving)	1.2	1.3	1.3	1.3	1.3
Ethyl mercaptan (added before autoclaving)	1.2	1.5	1.6	1.7	1.9
Control media (2) (sulfate)	1.0	.....	.....	4.8	.....

left sterile. All were incubated at 28°C. for 5 weeks; the nitrogen was determined by the Kjeldahl method, and the sulfate sulfur, by the Woodman and Evans method (6). The number of milligrams of nitrogen fixed in the presence of colloidal sulfur and various sulfur-carrying compounds are given in table 1. Each reported result is the average of three or more closely agreeing determinations.

Since ethyl mercaptan and mustard oil are volatile, they were added to some flasks before autoclaving and to others after autoclaving. The gain in nitrogen in the presence of the heated and non-heated ethyl mercaptan was low, indicating the unavailability of the sulfur.

When the mustard oil was added after autoclaving there was no growth at the higher concentrations and little or none at the lower concentrations, thus indicating that mustard oil is toxic. When mustard oil was added before autoclaving, considerable growth occurred but no fixation. In this case it appears that the mustard oil was hydrolyzed, the resulting compounds not being toxic; however, nitrogen fixation was discouraged because of the combined nitrogen. Traces of sulfur in the chemicals were no doubt sufficient for the small gains in nitrogen found in the checks (those receiving no sulfur). There was slight early growth in the checks, which ceased about the fourth or fifth day of incubation. No nitrogen was fixed in the presence of sodium thiocyanate or sodium sulfanilate. This was not due to toxicity, but the combined nitrogen discouraged fixation by *Azotobacter chroococcum*. This is shown by the profuse growth in the medium to which these compounds were added; when the nitrogen was determined the quantity found, however, was no greater than that added in the sulfur-carrying compound. Although the cysteine supplied combined nitrogen it did not interfere with nitrogen fixation. The gain in nitrogen was slightly less in the presence of 150 p.p.m. of cysteine over that in the presence of smaller quantities. No fixation occurred, nor was there appreciable growth, in the presence of sodium sulfocarbolate, sulfur iodide, and sulfonmethane. No growth appeared in the presence of sulfur iodide, apparently because of its toxicity; whereas scant growth occurred in the presence of sodium sulfocarbolate and sulfonmethane, apparently because of the unavailability of sulfur in these compounds.

Sodium thiocarbonate, sodium ethyl sulfate, colloidal sulfur, sulfur chloride, sulfuryl chloride, sodium sulfide, sodium sulfite, and sodium sulfate are all valuable carriers of sulfur to *Azotobacter chroococcum*, judged from gains in nitrogen occurring in their presence. The efficiency of the different sulfur carriers varies slightly with the concentration. Apparently, 5 p.p.m. of sulfur in any of the carriers was sufficient to meet the needs of *Azotobacter chroococcum* under these conditions. None of the sulfur carriers were toxic at 100 p.p.m.

The sulfates present in the sterile and in the inoculated media at the close of the incubation period were determined, and average results are given in table 2.

No sulfate was found in the sterile or in the inoculated media to which had been added mustard oil, sodium sulfocarbolate, sulfur iodide, sulfonmethane, sodium sulfanilate, and ethyl mercaptan. There were no gains of nitrogen in the media containing these sulfur compounds, indicating that the absence of sulfates predicates the absence of nitrogen fixation by *Azotobacter chroococcum*. At the end of the incubation period the sterile media to which had been added sodium thiocyanate, sodium ethyl sulfate, colloidal sulfur, sulfur chloride, sulfuryl chloride, sodium sulfide, sodium sulfite, and cysteine carried from 8 to 100 per cent of the added sulfur as sulfates. As nitrogen fixation progressed, the sulfates disappeared; they disappeared completely from the media containing sodium thiocyanate, colloidal sulfur, and cysteine hydrochloride. When

mustard oil was added before autoclaving, sulfates appeared; when added after autoclaving, no sulfates appeared. A comparison of results in tables 1 and 2 shows that in those media in which sulfates appeared there were gains in nitrogen, whereas in those in which sulfates did not appear there were no gains in nitrogen. An exception was sodium thiocyanate; here the combined nitrogen apparently discouraged fixation.

From these data it appears that *Azotobacter chroococcum* requires sulfur in the form of sulfates, and all compounds which are spontaneously oxidized to sulfates or compounds which are converted by bacterial activities into sulfates may serve as valuable sources of sulfur. The limited number of compounds tested points to the conclusion that *Azotobacter chroococcum* can neither oxidize sulfur and sulfur-carrying compounds to sulfates nor use other compounds of sulfur.

TABLE 2

Percentages of original sulfur recovered as sulfates from incubated media to which had been added 50 p.p.m. of colloidal sulfur and different sulfur-carrying compounds

	PROPORTION OF ORIGINAL SULFUR RECOVERED AS SULFATES	
	Not inoculated	Inoculated
	per cent	*per cent*
Mustard oil	0	0
Sodium thiocyanate	12.9	0
Sodium thiocarbonate	76.4	47.8
Sodium sulfocarbolate	0	0
Sodium ethylsulfate	100.0	98.6
Colloidal sulfur	8.0	0
Sulfur chloride	52.5	26.4
Sulfuryl chloride	93.7	89.6
Sulfur iodide	0	0
Sulfonmethane	0	0
Sodium sulfanilate	0	0
Sodium sulfide	66.5	52.8
Sodium sulfite	100.8	96.2
Cysteine hydrochloride	35.5	0
Ethyl mercaptan	0	0

SUMMARY

Azotobacter chroococcum was cultured in a basic nutrient medium to which were added colloidal sulfur and various sulfur-carrying compounds in varying quantities. Because of the toxicity of the compound, *Azotobacter chroococcum* did not grow in the presence of mustard oil and sulfur iodide, even when 5 p.p.m. were added. No nitrogen was fixed in the presence of sodium sulfocarbolate and sulfonmethane, because of the unavailability of sulfur, and none was fixed in the presence of autoclaved mustard oil, sodium thiocyanate, and

sodium sulfanilate, not because of toxicity, but because of the combined nitrogen content of the compounds, which discouraged nitrogen fixation. Cysteine supplied combined nitrogen, yet it did not discourage nitrogen fixation.

Sodium thiocarbonate, sodium ethyl sulfate, colloidal sulfur, sulfur chloride, sulfuryl chloride, sodium sulfide, sodium sulfite, and sodium sulfate all are valuable carriers of sulfur to *Azotobacter chroococcum*. Judged from the gains in nitrogen occurring in their presence, apparently *Azotobacter chroococcum* requires sulfur in the form of sulfates and all compounds which are spontaneously oxidized to sulfates or compounds which are converted by bacterial activities into sulfates may meet the sulfur requirements of *Azotobacter chroococcum*.

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THE EFFECT OF FERTILIZERS AND LIMING UPON THE ELECTRODIALYZABLE MANGANESE OF SASSAFRAS SILT LOAM[1]

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Since the soil is a dynamic system in which the equilibrium is constantly undergoing changes brought about by such factors as hydrogen-ion concentration, moisture, temperature, bacterial action, removal of ions by plants and their addition by fertilizers, the effects of subjecting a soil to a definite program of fertilizers and cropping over a long period should be reflected in the nature and amount of ions adsorbed by the soil.

During the course of an investigation of the effect of fertilizers, liming, and cropping on the electrodialyzable bases of Sassafras silt loam, the results obtained for manganese appeared to be of sufficient interest to warrant their publication prior to completion of the investigation.

REVIEW OF LITERATURE

Schollenberger and Dreibelbis (14) while investigating the effect of cropping with various fertilizers, manure, and lime treatments upon the exchangeable bases of plot soils found no consistent indications of any very marked influence on the exchangeable manganese by fertilizers. The manure plots were notably high in exchangeable manganese; the most acid of the fertilizer plots, however, was the highest.

Davidson (3) employed water cultures to determine the effect of different ions on the absorption of manganese by wheat seedlings and found that the availability of manganese was governed by the combined effect of hydrogen-ion concentration and the anions present. Absorption was inhibited by phosphates but was greater with chlorides and sulfates than with the oxides.

Mann (7) found that manganese absorbed by plants exhibited a curve which closely followed that of the solubility of this element in the soils.

Willis (16, 17, 18) cautions against the use of the absorption by plants as an index of availability of manganese on account of the physiological interference of calcium compounds and stresses the importance of placing the factor "oxidation-reduction potential" on a quantitative basis.

Smolik (15) concluded that all soil manganese is reduced to manganese capable of exchange with simultaneous increase in pH of the soil.

Manganese deficiency, which is often induced by liming, may be corrected by the addition

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of soluble manganese salts (6, 17), manure (5, 13, 14), or physiologically acid fertilizers (1, 4, 6, 8, 9).

EXPERIMENTAL

Treatment of plots

The plots from which the samples were taken for the investigation are designated as "Block B" of the soil plots at the experiment station farm. The soil is classified as Sassafras silt loam.

The treatments to which the plots were subjected over a period of 24 years are indicated in table 1. Each plot (1/10 acre) was divided into two sections, one of which received lime as follows: 1908, 1916 at the rate of 2000 pounds per acre; 1912, 1920, 1924 at the rate of 1500 pounds per acre. The last

TABLE 1

Treatment of plots, 1908–1932

Total in pounds per acre

PLOT NUMBER	MURIATE OF POTASH	SODIUM NITRATE	DRIED BLOOD	SUPER PHOSPHATE	FARM MANURE
					tons
4	1,335	.....	.....	.....	.....
6		Check			
7	1,335	.....	.....	3,900	.....
8	1,335	1,675	.....	.....	.....
9	1,335	1,599	75	3,900	.....
10	2,550	3,150	100	7,300	.....
11		Check			
14	.....	.....	.....	750	65
15	.....	.....	.....	.....	130
16		Check			

application of lime was made in 1924. Both the limed and unlimed sections received the same fertilizer and manure treatments.

The following cropping system was employed on the plots from 1908–1912: corn, oats, wheat, and timothy + clover; from 1912, it has been corn, soybeans, wheat, and timothy + clover.

Sampling

Samples were taken in the late summer of 1932. Twenty cores ($1\frac{5}{8}$ inch x $6\frac{2}{3}$ inch) were taken from each section (limed and unlimed) of each plot with the utmost care to insure samples as representative as possible. Each sample was thoroughly mixed, air-dried, and again quartered.

Electrodialysis

One hundred grams of the air-dried soil was electrodialyzed in a three compartment Mattson cell[3] using parchment membranes and perforated platinum

[3] As listed by Arthur H. Thomas Company, Philadelphia, Pennsylvania.

sheet electrodes. With this arrangement the electrodes were approximately 2 cm. apart. Current was applied from a motor generator at 110 volts. The soil was subjected to electrodialysis for 1 hour, the dialyzates were removed, and electrodialysis was continued for three 8-hour periods, making a total of 25 hours for each sample.

TABLE 2

Effect of fertilizers and liming upon total and electrodialyzable manganese of Sassafras silt loam

M.E. manganese (as Mn) per 100 gm. dry soil*

PLOT	TREATMENT	TOTAL	DIALYZED	PER CENT DIALYZED	pH
4 L	K	3.06	0.582	19.02	6.72
4 NL		2.47	0.164	6.64	5.28
6 L	Check	2.44	0.618	25.33	6.92
6 NL		3.28	0.334	10.18	5.43
7 L	PK	2.44	0.674	27.62	6.87
7 NL		3.28	0.503	15.33	5.51
8 L	NK	2.39	0.528	22.09	6.71
8 NL		3.53	0.255	7.23	5.68
9 L	NPK	2.55	0.429	16.82	6.85
9 NL		3.53	0.205	5.80	5.56
10 L	2(NPK)	2.47	0.703	28.46	6.63
10 NL		3.31	0.559	16.89	5.59
11 L	Check	2.91	0.706	24.26	6.93
11 NL		2.88	0.370	12.84	5.42
14 L	P + Manure	3.57	0.900	25.21	6.80
14 NL		2.95	0.646	21.89	5.47
15 L	2 Manure	3.57	0.760	21.28	6.64
15 NL		3.24	0.550	17.00	5.53
16 L	Check	2.94	0.615	20.92	6.70
16 NL		3.20	0.529	16.53	5.44

* 18 hours at 110°C.
L, limed. NL, Not limed.

Results

The course of the current was remarkably uniform throughout the series, rising to a maximum of approximately 300 milliamps within a few minutes and decreasing gradually as dialysis progressed until it became practically constant at 30 milliamps. Analyses of the dialyzates are presented in table 2.

The pH measurements were made after the soils had stood with occasional shaking for 3 days in 2.5 times their weight of water. The glass electrode (MacInnes-Dole type) with electron-tube potentiometer was employed.

As indicated in table 2, electrodialysis removed a greater amount of manganese from the limed plots without exception. The fertilizer plots and the checks (except 16) show the greatest percentage variation between the limed and the unlimed sections. The percentage yield for manganese was lowest in plots 4 and 9 (limed), and 4, 8, and 9 (K, NK, and NPK respectively) of the unlimed plots. The manure plots were high in dialyzable manganese.

With the exception of plots 4 (KCl), 11 and 16 (checks), 14 (P+manure), and 15(manure), the limed plots contained less total manganese than the unlimed plots.

Although the pH of plot 4 (KCl only) is the lowest, there is no marked variation within each group brought about by the treatments. The effect of liming on the reaction of the soils is apparent.

DISCUSSION

If the amount of manganese removed by electrodialysis is assumed to be an index of the availability of this element to plants it will be observed that the foregoing results are in conflict with the conclusions drawn from the results obtained by numerous investigators, i.e., that the availability of manganese decreases with decreasing hydrogen-ion concentration and is reduced to a low level by liming, for without exception the plots receiving lime, and consequently with the lower hydrogen-ion concentration, yielded more manganese than did the unlimed plots.

Since it has been shown (11) that electrodialysis and neutral salt solutions extract essentially equivalent amounts of bases from soil colloids (and it is reasonable to assume that the action on whole soils would be comparable) and since Schollenberger and Dreibelbis (14) employed neutral salt solutions and found that the manure plots were notably high but that the most acid of the fertilizer plots was the highest in exchangeable manganese, it is logical to assume that the amount of manganese removed by electrodialysis could be employed as an index of availability of this element in the plots under consideration. Analyses of samples of mixed hay from plots 10 and 11 showed that hay from the limed sections contained very little manganese whereas that from the unlimed sections contained appreciable amounts. The foregoing assumption is therefore unjustified.

The most obvious explanation of the results reported would be that as electrodialysis progressed the pH of the system was lowered with resulting increase in availability of manganese. If liming decreases the availability then a conservation of manganese should be reflected in the total manganese of the limed plots, yet the analyses indicate that in several of the limed sections manganese is lower than in the corresponding unlimed sections. Undoubtedly some factor or factors other than reaction alone influence the availability of

manganese, for it has been found that in some cases manganese is readily available at hydrogen-ion concentrations well above neutrality.[4] It was also found that chlorosis, which occurred during the summer growing season and which was corrected by the addition of manganese sulfate, did not occur on the same plots during cool, wet weather (6, 19).

These results and those of Mann (10) and Willis (16, 17, 18) suggest the possibility of an enhancement of the reduction of manganese by water-logging of limed soils.

Shortly after the plots were laid out it was found necessary to drain a section of the blocks. The tile crosses plots 15 and 16 diagonally. In 1928, lateral drains were laid through both sections (limed and unlimed) of each block.

Analyses of mixed hay from these plots indicate that the plot soils react normally as regards manganese during the hot, comparatively dry season. Therefore, the loss of manganese from the limed sections (which cannot be attributed to increased yield alone) must be caused by greater leaching from the limed than from the unlimed soil during the cold, wet months.

Although the plots were not laid out with studies of this character in mind, they furnish evidence of the effects of variations in physical condition of the soil upon its chemical character. A check on the manganese status of these plots some years hence should reflect the effect of draining the remainder of the plots.

Unfortunately, samples of these soils which were taken when the plots were established have been lost, and analyses which would be of value in establishing the original status of manganese are not available.

SUMMARY

Samples from a series of soil plots which had been subjected to a definite schedule of treatments during a period of 24 years were electrodialyzed in order to determine the effect of these treatments upon the amount of manganese removed by this method.

Without exception, the limed soils yielded the greater amount of manganese. On a percentage basis plots 4 and 9 of the limed group, and plots 4, 8, and 9 (K, NK, and NPK respectively) of the unlimed group yielded the least.

Analyses of mixed hay indicated that very small amounts of manganese were removed from the limed plots, whereas appreciable amounts were removed from the unlimed, yet certain limed plots contained less total manganese than did the corresponding unlimed plots.

The results indicate the possibility of greater leaching from the limed plots during the cold, wet months, which may be attributed to the enhancement of reducing conditions brought about by water-logging of limed soils.

Although the KCl unlimed plot had the lowest pH, there was no marked variation within each group resulting from the treatments.

[4] From unpublished data of Dr. T. G. Phillips, N. H. Agricultural Experiment Station, Durham, New Hampshire.

No consistent indications of any marked influence of fertilizers on the electrodialyzable manganese of the plot soils were observed.

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SORPTION IN AN IDEAL SOIL

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The ideal soil is an assemblage of spheres packed at random; it possesses a structure which, if the spheres are not too large, renders it an absorbent. It may accommodate masses of liquid condensed from the vapor state into capillary bodies; i.e., capillary condensation. It may, depending on the grain size, also offer an internal surface sufficiently large to hold an appreciable amount of liquid and render it an adsorbent as well; the molecules of the sorbed vapor are held in the form of an adsorbed layer, generally monomolecular, which extends over the available surface of the grains. Thus if into a quantity of soil, initially dry, vapor is introduced at a pressure below that required for saturation, a certain fraction of the molecules will condense into capillary masses of liquid enmeshed in the packing and the balance will be adsorbed on the parts of the total grain surface not covered by these masses. We shall first consider the capillary condensation of vapor in the soil.

The capillary surfaces possible to the condensed liquid masses are, in many respects, similar to those existing after drainage of an initially saturated soil when equilibrium has been established. In either case the physical processes involved are subject to the laws of thermodynamic equilibrium between a liquid and its vapor when separated by a capillary interface. Elsewhere (8) it has been shown that these capillary bodies may be simple rings of liquid around contact points, webs of liquid extending over two or more contacts, surfaces confining small masses of liquid which saturate the pore space of several neighboring grains, or, finally, the extended capillary surface present when saturation exists as in the case of capillary rise. In a column of soil sufficiently short, so that gravity effects are negligible, only one of these surface types will be found; if, however, the column is long, then part or all, depending upon its length and the maximum vapor pressure prevailing, may exist. The behavior may be better understood by following the hydration of a small sample of an initially dry soil.

We suppose a sample of soil sufficiently small that the liquid distribution is homogeneous and not affected by gravity, and follow the capillary condensation from zero vapor pressure to that prevailing over a plane surface. We suppose, first, that the vapor is introduced at very small vapor pressure. The

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condensed vapor must accumulate at the grain contact points and form rings of liquid wrapped around the axis of each pair of grains; otherwise the energy of the system would not be a minimum. If the vapor pressure is increased to a new value, more vapor must be supplied to maintain equilibrium; the additional vapor will condense on the rings already formed and increase their size. As the vapor pressure is further increased, the process of growth will continue until the rings overlap and merge. The boundary is then no longer capable of sustaining, everywhere, a distribution of single rings. A second type of capillary mass begins to exist, a web of liquid involving two or more contacts (the packing is not a regular array) and formed by the coalescence of several rings. These transformations are illustrated in figure 1. As the vapor pressure is still further increased, these masses likewise grow until they combine; the whole pore space becomes completely filled with liquid. There are, thus, for capillary condensation in the soil, three distinct stages of liquid distribution in the hydration process, each corresponding to a definite range of vapor

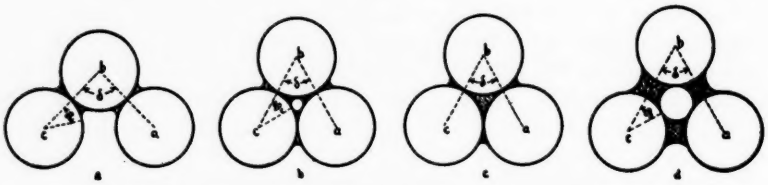

FIG. 1. TRANSFORMATION OF SIMPLE LIQUID RINGS

(a) Single rings with angle $\delta > 60°$; (b) Single rings about to merge in three grain pore with $\delta = 60°$; (c) Simple web of liquid existing after coalescence of rings; (d) Rings of liquid in spaced hexagonal packing, with $\delta = 60°$.

pressures; at low vapor pressures the distribution consists of simple rings—the pendular stage (8). The succeeding or funicular stage is a stage of coalescence of the simpler forms and is characterized by the presence of webs of liquid. There is, finally, at vapor pressures near that over a plane surface, a stage of complete saturation.

The bounding surfaces of the condensed liquid masses are capillary and when at equilibrium possess definite curvatures which can be determined from the vapor pressures by the Kelvin equation (10). We shall use the following integral form of this equation:[1]

$$\int_S dS \cos\phi[(p - p_0) - (\rho p_0/D_0) \log_e(p/p_0)] = \sigma \int_s \cos\theta \, ds \qquad (A)$$

The integrals extend, respectively, over the capillary surface S and around its bounding contour s. ϕ is the angle between the normal to the surface-element dS and the vertical; ds is an element of the meniscus-solid contour;

[1] See Appendix 1.

and θ is the angle between the vertical and that normal to ds which is tangent to the meniscus-surface. p_0 is the vapor pressure just over the free liquid surface, and p is the vapor pressure over the element dS. ρ and D_0 are, respectively, the densities of the liquid and vapor phases, and σ is the surface tension of the liquid.

It is permissible, for most purposes in soil physics, to neglect the differential variation in vapor pressure and to consider the vapor pressure constant over the meniscus; this is because such variations are due to gravity and are not in excess of the disturbance which would arise from a distance of one grain radius. With constant surface tension σ, we find, if a is the area of projection of the meniscus-surface on the horizontal, that

$$(p - p_0) - (\rho p_0/D_0) \log_e(p/p_0) = \left(\sigma \int_s \cos\theta \, ds\right) \Big/ a \qquad (B)$$

There are two additional approximations[1]

$$\frac{(\rho - D_0)}{D_0} (p - p_0) = \left(\sigma \int_s \cos\theta \, ds\right) \Big/ a \qquad (C)$$

and

$$-(\rho p_0/D_0) \log_e(p/p_0) = \left(\sigma \int_s \cos\theta \, ds\right) \Big/ a \qquad (D)$$

Equation (C) is applicable only for vapor pressures near that of a plane surface. Equation (D) corrects for the compressibility of the vapor and is usually sufficient for most practical purposes; it enables us to find the vapor pressure over a capillary surface in equilibrium when the liquid-solid contour is known. We have then

$$p = p_0 e^{-(\sigma D_0/\rho p_0)\left(\int_s \cos\theta \, ds\right)/a} \qquad (E)$$

Complete wetting will be assumed, and equations (B, C, D and E) will be applied to a uniform ideal soil, that is, one composed of identical spherical grains packed at random. The mass of the vapor absorbed under any given vapor pressure p and held as liquid by capillary condensation will, where possible, be estimated.

When vapor is first introduced into a dry soil, the distribution is pendular, and the capillary-condensed liquid is in rings around the grain contacts. The approximate volume of a single ring is easily found and is given by[2]

$$(v/2\pi r^3) = (\sec\theta - 1)^2 [1 - (\pi/2 - \theta) \tan\theta] \qquad (F)$$

[1] Compare (8). Equation (F) was first obtained by Haines and Fisher; see also the derivation and references given by E. Manegold (6).

where r is the grain radius. θ is the size of the ring—cf. fig. 2a—and is defined by the angle θ included between the line of centers of the two grains support-

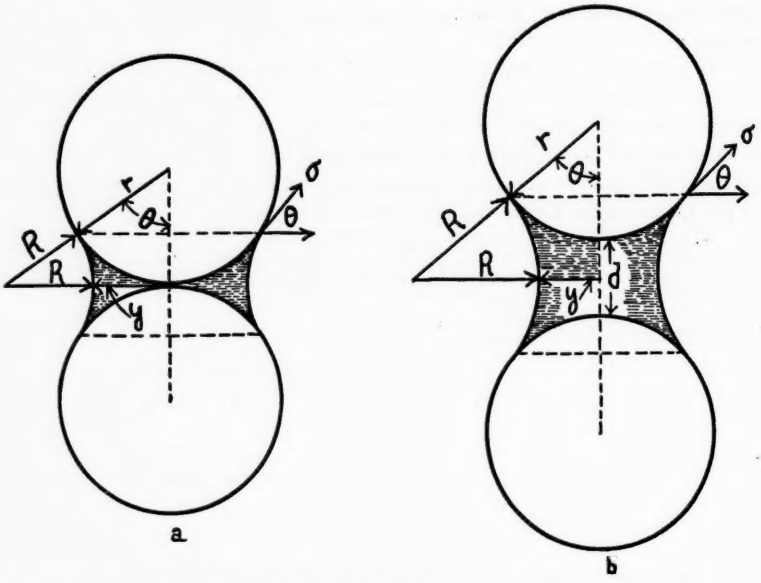

FIG. 2. (a) SIMPLE PENDULAR RING OF LIQUID. (b) SIMPLE RING OF LIQUID IN SPACED HEXAGONAL PACKING

ing the ring and the radius vector from the center of one of the grains to the outer edge of the ring on that grain (8). Setting

$$\lambda = -(\rho\, p_0/\sigma\, D_0) \log_e(p/p_0) \qquad (G)$$

we find on applying equation (D) to a simple ring that

$$\lambda = \frac{2(2\pi r \sin\theta)\cos\theta}{(2r - 2r\cos\theta)\, 2\pi r \sin\theta}$$

from which

$$\sec\theta = 1 + \frac{1}{r\lambda} \qquad (H)$$

We find from equation (F) and (H)

$$v/(2\pi r^3) = (1/r\lambda)^2 \{1 - [\sin^{-1} 1/(1+1/r\lambda)]\, [(1/r\lambda)\,(2 + 1/r\lambda)]^{\frac{1}{2}}\} \qquad (I)$$

as the volume of liquid held by two grains of radius r at their common contact in equilibrium under a vapor pressure p. The value of λ is obtained from

equation (G) from the known values of ρ, σ, D_0, p, and p_0. A more accurate expression is obtained by use of equation (B). We set

$$\lambda' = (p - p_0) - (\rho\, p_0/\sigma\, D_0) \log_e (p/p_0) \qquad (J)$$

and obtain

$$\sec \theta = 1 + \frac{1}{r\lambda'} \qquad (K)$$

An equation for $(v/2\pi r^3)$, identical with equation (I), is obtained from equations (F) and (K), except that λ is replaced by λ'.

To find the total quantity of liquid in the pendular region, we must know the total number of such rings in each cubic centimeter of packing. The number of contacts (two grains per contact) per cubic centimeter of packed space is equal to the number of rings existing in the same region and has been shown, elsewhere (8), to be given by

$$N = (3/(8r^3))\,(1 + 1.828\,x) \qquad (L)$$

where $x = (0.476 - P)/0.217$, P being the porosity of the packing. The total volume of retained liquid Ω_p in 1 cc. of packed space is then

$$\Omega_p = Nv \qquad (M)$$

where N is found by equation (L), and v, by equation (I). The total quantity of liquid held in a volume V is $(\Omega_p V)$. The relative retention is defined as the ratio of the liquid actually retained to that required to saturate the pore space; it is expressed, in the pendular region, by

$$\Omega_p/P = Nv/P \qquad (N)$$

The mass of the absorbed vapor is easily found from equation (M) provided the densities of the liquid and its vapor, at pressure p, are known.

The pendular region is characterized by the presence of a ring distribution throughout; its bounding vapor pressures can be easily established. The ring size is governed by the vapor pressure [equation (H)], and all rings from zero radius up to the radius prevailing just before the first coalescence are permissible. The value $p = 0$ defines the lower limit of the zone and corresponds to the ring of zero radius. Coalescence first appears in the smallest configuration of the packing—three grains, in contact, whose centers form an equilateral triangle (8). The angle between the axes of two adjacent rings in this configuration is 60°; hence the critical value of θ is 30°. Therefore, from equation (H), the rings first merge, during hydration, when

$$r\lambda = 6.464 \qquad (O)$$

The critical vapor pressure p_c is obtained by solving equation (G) with $\lambda = 6.464/r$;

$$p_c = p_0 e^{-\frac{\sigma D_0 (6.464)}{r \rho p_0}} \qquad (P)$$

The same result can be obtained from equation (E) with $\theta = 30°$. Equation (P) thus fixes the vapor pressure defining the junction of the funicular and pendular regions. All values of p such that $0 < p < p_c$ will, when the packing is hydrated, result in a ring distribution throughout.

The funicular region is the zone of coalescence; coalescence not only of single rings but of more complicated forms. It commences when the pendular rings begin to merge and ends when saturation first appears. Conditions are more complicated, and several types of liquid configuration may exist. There may be single rings, for there are, in the ideal soil, many pairs of adjacent grains whose axes make an angle greater than 60° (fig. 1a); θ may be greater than 30°, and rings larger than those in the pendular region may exist. There may also exist single webs of liquid formed by the coalescence of two or more rings; for example, the web existing in the pore cell of three adjacent grains, in contact, whose centers form an equilateral triangle, and formed by the coalescence of the rings originally existing at its contacts (fig. 1c). And, finally, there may be single cell-configurations completely saturated, because of the mergence of several neighboring rings or webs of liquid; for example, the regular tetrahedron, formed by the centers of four neighboring grains, will be completely filled when the rings associated with it merge.

An exact solution of the problem, in the funicular region, is difficult, and depends on a rigorous analysis of the packing problem. We shall only attempt to calculate a lower limit for the amount of liquid which can be retained when the funicular region is hydrated. As an approximation we shall resort to the statistical scheme of packing in which the grains are arranged in regular hexagonal array and spaced at a distance $(2r + d)$ where d is a spacing constant adjusted to suit the porosity (8). We shall regard the liquid as distributed in the form of rings between each pair of grains of the stretched packing. This distribution represents the minimum possible in the hexagonal soil. The volume of the pore space occupied by the rings is certainly less than that taken up by the complicated masses present in an actual soil of the same porosity.

The size of the funicular ring is defined as before (fig. 2b). The angle θ of the new ring is found, from equation (D), to be given by

$$q \sec \theta = 1 + \frac{1}{r\lambda} \qquad (Q)$$

where λ is defined by equation (G) and q is defined by the equation $q = (2r+d)/2r$. q is found to be given in terms of the porosity by the equation (8)

$$q = 0.9045/(1-P)^{1/3} \qquad (R)$$

The volume of the ring is given by the expression (8)

$$v/(2\pi r^3) = q(q \sec\theta - 1)^2[1 - (\pi/2 - \theta)\tan\theta] - (1/3)(q-1)^2(q+2) \qquad (S)$$

From equations (Q) and (S) we find

$$v/(2\pi r^3) = (1/r\lambda)^2 \{q - [\sin^{-1} q/(1 + 1/r\lambda)][(1 + 1/r\lambda)^2 - q^2]^{\frac{1}{2}}\} - (1/3)(q-1)^2(q+2) \qquad (T)$$

Equation (J) may give a more accurate value; the resulting equations are identical with equations (Q) and (T) except that λ is replaced by λ' throughout. Equation (T) determines the volume of a "funicular" liquid ring in equilibrium under the vapor pressure p. λ is determined from equation (G) from the known values of ρ, σ, D_0, p, and p_0. The total number of "funicular" rings per cubic-centimeter of packed space is given, as in an earlier paper (6), by

$$N_f = 9(1 - P)/(2\pi r^3) \qquad (U)$$

The total volume of liquid Ω_f retained in 1 cc. of packed space is therefore

$$\Omega_f = N_f v \qquad (V)$$

where v is given by equation (T), and N_f, by equation (U); the value of q is found from equation (R). The retention in a volume V of packing is $(\Omega_f V)$. The relative retention is

$$\Omega_f/P = N_f v/P \qquad (W)$$

The funicular masses, as the vapor pressure is increased, will grow until they coalesce and the packing becomes saturated. The funicular rings of the spaced hexagonal packing will merge when $\theta = 30°$ (Fig. 1d), and since the packing is symmetrical all must coalesce simultaneously, with resultant saturating of the packing. The small distortions required to transform the hexagonal soil, with grains separated by a distance $(2r + d)$, into an ideal soil, with random piling, will not alter this behavior to any degree; hence during hydration, both packings, if their porosities are equal, become saturated at the same pressure. We can, therefore, determine the vapor pressure p_s at which complete saturation occurs when the soil is hydrated; with $\theta = 30°$ we find from equations (Q) and (R) that

$$\frac{1}{r\lambda} = \frac{1.044}{(1 - P)^{1/3}} - 1 \qquad (X)$$

and the critical pressure, that at which complete saturation first occurs during hydration, is given by

$$p_s = p_0 e^{-\frac{\sigma D_0}{r\rho p_0}\{1/[1.044/(1-P)^{1/3}-1]\}} \qquad (Y)$$

All values of p such that $p_c < p < p_s$ lie in the funicular region; p_c and p_s are the limiting vapor pressures.

The quantity of liquid held per cubic centimeter of packing in the saturation region is approximately equal to P, the porosity, and the total in a volume V of packing is (VP).

After the packing becomes saturated, and the prevailing vapor pressure p becomes greater than the saturation pressure p_s, the only possible capillary

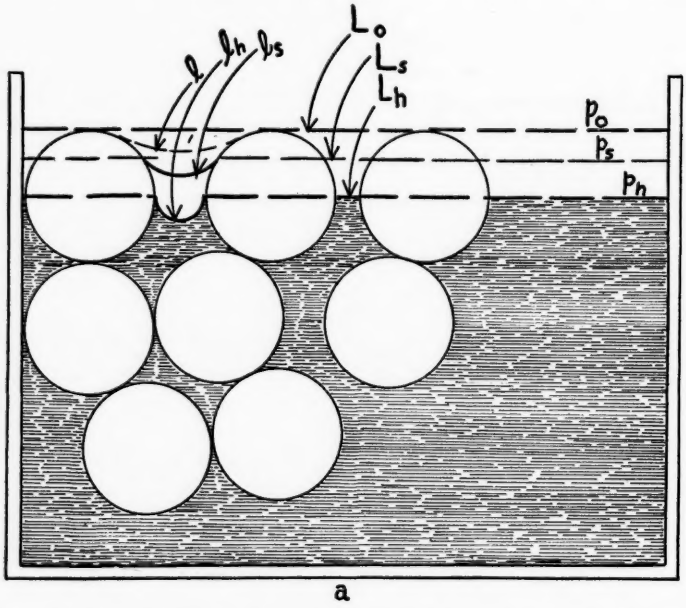

FIG. 3. POSSIBLE POSITIONS, IN THE BOUNDING GRAIN LAYER, OF A CONTINUOUS MENISCUS ENCLOSING A SATURATED PACKING

l_h is a section of the meniscus between two grains when it occupies the plane of centers, L_h, at the vapor pressure p_h; l_s, L_s, and p_s are similar quantities for the minimum rise position; L_0 and p_0 are for a plane surface; l marks a section of a meniscus intermediate to L_0 and L_h; (a) packing with plane boundary; (b) packing with spherical boundary.

surface is that extending over the outer layer of grains bounding the packing; the packing remains saturated except in the bounding grain layer. If, for example, the soil is contained in an open cup, the capillary surface extends across the top layer of grains as in figure 3a. Any further increase in vapor pressure results in condensation on this outer boundary until the Kelvin relation is satisfied. L_s, in figure 3a, is the plane of the saturation meniscus when $p = p_s$; L_0 is the surface commonly tangent to the spherical grains and corresponds to the vapor pressure $p = p_0$, that over a plane surface of the liquid;

the maximum volume of additional liquid that can be condensed is closely that held between L_s and L_0. The outer grain layer can accommodate all curvatures between that of minimum capillary rise, corresponding to p_s, and zero, that of a plane surface for p_0; the vapor pressure may, therefore, vary from p_s to p_0, while the amount of liquid condensed does not exceed the volume occupied by the grain layer on the outer boundary, and is, in the ordinary packed sample, a very small amount. Figure 3b shows the corresponding relations in

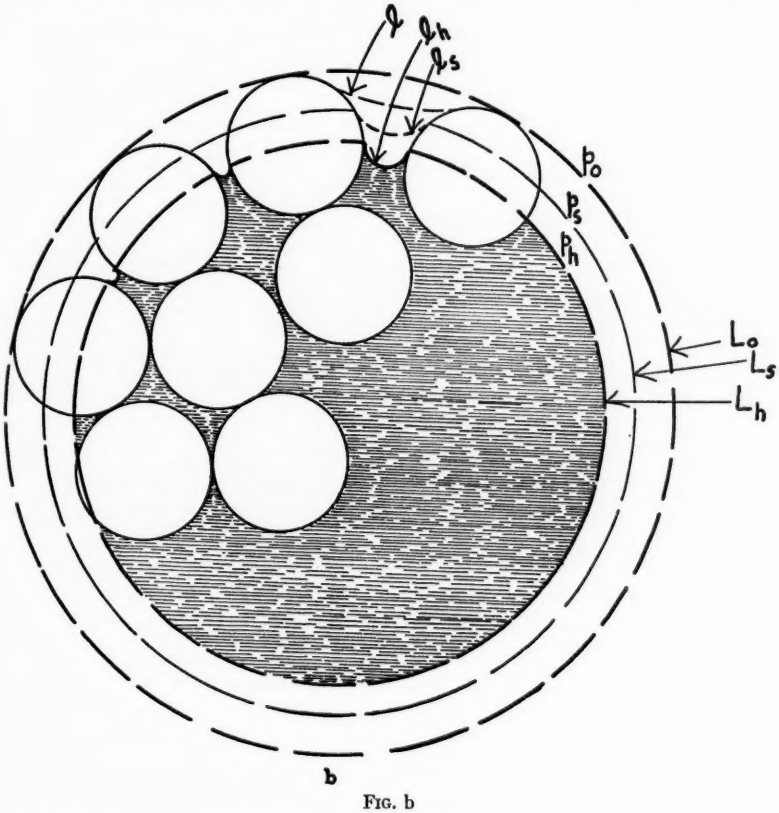

FIG. b

an isolated sample of soil with spherical boundary; the circles L_s and L_0 enclose the region in which condensation may occur when $p > p_s$. It must be recalled that the samples represented in figures 3a and 3b are small, and condensation, since the vapor pressure is approximately constant throughout, occurs uniformly through the whole sample; a saturation meniscus must then lie in the outer boundary.

Figure 4a shows the relation between the relative retention and the vapor pressure p. The curve $0_0 0_c 0_f 0_2 0_3$ is that of hydration. $0_0 0_c$ defines the pendu-

lar region [computed from equation (*N*)]; $O_c O_f$, the funicular, and is a limiting curve [computed from equation (*W*)]. The straight portion $0_f 0_2$ marks the passage from the funicular to the saturation region, under the constant vapor pressure p_s [computed from equation (*Y*)]; it is, likewise, a limiting curve. The hydration curve for an actual soil will lie beneath $0_f 0_2$; the curve corresponding to $0_0 0_f 0_2$ will lie within the "loop" of figure 4b. Complete saturation first occurs at 0_2 (neglecting the error introduced by the bounding grain layer). $0_2 0_3$ corresponds to the saturation region, just discussed, where $p_s < p < p_0$.

If, now, the packing is dehydrated, the hydration curve, for the most part, is not retraced. We shall follow the process. We suppose the soil sample initially saturated under a vapor pressure p_0; the meniscus is then in the extreme edge of the outer grain layer, L_0 of figures 3a and 3b. The outer boundary is

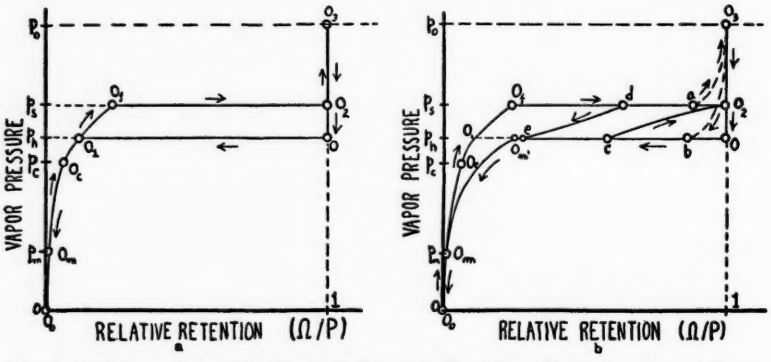

FIG. 4. SCHEMATIC CURVES SHOWING THE RELATIVE RETENTION REPRESENTED AS A FUNCTION OF THE VAPOR PRESSURE—THE SORPTION CYCLE

(a) Simple sorption cycle showing saturation hysteresis; (b) the distortions produced by microscopic hysteresis and the presence of a free gas in the packing, together with the effects produced by reversing, in a partially saturated packing, either hydration or dehydration.

capable of sustaining a meniscus until the latter occupies the position of maximum capillary rise, in which position the meniscus threads through the plane of centers of each cell (9). All curvatures from that of a plane surface for a vapor pressure p_0 down to that prevailing at maximum capillary rise for a vapor pressure p_h can be accommodated. Whether the meniscus comes from capillary rise or whether it forms by condensation, when removed, its behavior will, as far as curvature adjustments are concerned, be the same; it will remain in the layer of grains where it initially rests as long as its curvature can adjust itself to that layer. If, for example, a U-system is used (7, fig. 1) and liquid is allowed to rise into the soil contained in one arm of the U and thus reach equilibrium at minimum rise (7), then when the free liquid surface is lowered in the liquid arm of the U the meniscus will remain undisturbed, except for slight changes in the grain layer where it happens to be, until the distance between

the two, meniscus and free surface, exceeds the height of maximum capillary rise. Likewise if a soil sample is saturated and the vapor pressure over the meniscus is decreased, liquid will evaporate with an adjustment of the meniscus curvature in the grain layer where it happens to rest. The process will continue until a value of the vapor pressure p_h is reached beyond which curvatures not available in the packing, for a continuous meniscus, are required; this is the position of maximum capillary rise. Further decrease of vapor pressure means evaporation, and the continuous meniscus must break up. On dehydration, therefore, the packing will be saturated as the vapor pressure changes from p_0, that over a free surface, down to the value p_h when the meniscus runs through the plane of centers of each cell; the vapor pressure may change through a rather large range ($p_0 > p > p_h$) with a negligibly small loss of liquid. We observe that p_h the critical saturation pressure of dehydration is less than p_s the critical saturation pressure for hydration, for p_s corresponds to a meniscus intermediate to p_0 and p_h. These relations are shown in figures 3a and 3b. If, therefore, the packing is dehydrated commencing at the point 0_3 in figure 4a, corresponding to p_0 (for a plane surface), we pass, as the saturation pressure is decreased, through the point 0_2 corresponding to p_s the saturation pressure for hydration, and the curve $0_3 0_2$ is common to both hydration and dehydration. At 0_2 the curves of the two processes separate; the dehydration curve continues downward, with the packing saturated, until 0 the critical point of dehydration, with vapor pressure p_h, is reached. Values of p on the hydration curve, such that $p_s > p > p_h$, correspond to a discontinuous distribution of liquid masses; whereas those on the dehydration curve correspond to a saturated soil with the meniscus in the outer boundary. The value p_h is easily obtained; if we apply equation (E) to the boundary with the meniscus in the position of maximum capillary rise (9), we find for the critical saturation pressure of dehydration that[3]

$$p_h = p_0 e^{-\frac{\sigma D_0}{r \rho p_0} \frac{2}{[0.9590/(1-P)^{2/3}]-1}} \qquad (Z)$$

and for λ_h [equation (E) with $p = p_h$] we have

$$r\lambda_h = 2/\{[0.9590/(1 - P)^{2/3}] - 1\} \qquad (AA)$$

The continuous meniscus, when the vapor pressure is decreased below p_h, travels inward over the packing and disappears; discontinuous masses of liquid, broken off during the inward journey, then exist in the soil. The continuous meniscus may break up for either pendular or funicular vapor pressures, depending on the porosity of the packing. The values of equation (AA) range from $r\lambda_h = 4.20$, for a porosity $P = 0.48$ (porosities may be greater than 0.48),

[3] Equation (Z) is obtained by applying equation (E) to the spaced hexagonal packing; the value $(\int ds \cos \theta)/a$ is weighted according to the frequency of occurrence of the T, S, and R cells of the unit element (9).

to $r\lambda_h = 11.63$ for a porosity $P = 0.26$. When the value $r\lambda_c = 6.464$ [from equation (O)], which marks the junction of the pendular with the funicular region, is substituted in equation (AA), the corresponding porosity is found to be $P = 0.373$. Hence if $r\lambda_c < 6.464$, in which case $P > 0.373$, the saturation meniscus, on breaking up, leaves funicular liquid masses. If $r\lambda_c > 6.464$, in which case $0.373 > P > 0.26$, the saturation meniscus disintegrates in the pendular region. Referring to figure 4a, we expect the water content to decrease greatly along $0\ 0_1$, under a constant vapor pressure just less than p_h (the distance of $0\ 0_1$, from the Ω/p axis). $0\ 0_1$ should meet the hydration curve $0_0 0_c 0_f$ at 0_1 when the continuous meniscus has completely disappeared. We should then expect that, as the vapor pressure is further lowered, the funicular region $0_1 0_c$ would be retraced, thence the pendular region, 0_c, to the origin 0_0 where the soil is completely dried. This is not the case, however, for, because of the existence of microscopic hysteresis (8), neither the funicular region nor the part $0_c 0_m$ of the pendular curve is reversible.

Microscopic hysteresis is confined to configurations composed of a small number of grains. The random tetrahedron, for example, formed by the centers of four neighboring grains may for a given vapor pressure, suitably chosen, support either single rings at its contact points or a web of liquid, or finally it may be saturated; each capillary surface presents the same curvature. The Kelvin equation is satisfied with the tetrahedron subject to the same vapor pressure in each case. The amount of liquid retained in the tetrahedron is, however, different; when rings exist it is a minimum, and when the cell is saturated, a maximum.

The reason for the hysteresis lies in the fundamental difference of mechanism in the two processes. In hydration all liquid masses are formed by growth and coalescence of simpler forms; the amount of absorbed liquid must be a minimum. In dehydration, on the other hand, the discrete liquid masses are broken off the continuous meniscus as it moves through the interior of the soil, and left in the soil; after they are once placed in the packing, alteration is produced solely by evaporation. The relative sizes of the pore cells in a small volume of packing are of importance; a small pore may accommodate the curvatures necessary to a meniscus extending over it, whereas more open surrounding configurations may not. If, for example, we consider a close regular tetrahedron, formed by four neighboring grains in contact, and its surrounding grain configurations supposedly sufficiently more open than the tetrahedron, then the main capillary meniscus, as it moves through the compound configuration, will leave a capillary surface extending across each of the four pore cells bounding the tetrahedron while the more open pore cells surrounding it will be left empty since they are too large to support similar capillary surfaces; the tetrahedron will remain saturated as long as possible. Thus isolated liquid masses, each of which completely saturates the grain configuration supporting it, may exist during dehydration, whereas, during hydration, the identical configurations may be unsaturated for the same vapor pressure.

There is a definite limit to the size of these liquid forms. As the vapor pressure is decreased, the capillary surfaces bounding the web (for example, the liquid mass filling the interior of a three grain pore) recede farther into the supporting cell, until they meet in its plane of centers; each liquid mass must then break into rings. The extreme case occurs when the configuration is saturated; each bounding capillary surface may recede until it occupies the smallest opening of the cell (e.g., the plane of centers of the regular tetrahedron) beyond which it must break into rings.

The limiting vapor pressures of microscopic hysteresis may be easily fixed. The extreme configuration present in the random piling of the soil is a close regular tetrahedron with six actual contact points and outer faces composed of four three-grain pores. Each contact point is capable of supporting a ring until these merge when [equation (*G*)] $\lambda_c = 6.464/r$ and $p_c = p_0 e^{-6.464(D_0\sigma/r\rho p_0)}$. The tetrahedron may be saturated until the bounding capillary surfaces occupy the smallest opening in each of its faces; i.e., the plane of centers, the position taken during maximum capillary rise into the tetrahedron. The vapor pressure at which this occurs can easily be found; it is sufficient to consider a single face and apply equation (*D*). We find if λ_m is the value of λ at which it occurs that $\lambda_m = \pi r\sigma/(3^{\frac{1}{2}}r^2 - \pi r^2)$, or

$$\lambda_m = 19.5/r \qquad (BB)$$

Hence the limiting vapor pressure p_m is

$$p_m = p_0 e^{-19.5(D_0\sigma/r\rho p_0)} \qquad (CC)$$

Thus, during hydration, rings will exist in the tetrahedron when $\lambda_c < \lambda < \lambda_m$, whereas, during dehydration, it will be saturated in the same interval. When the vapor pressure becomes less than p_m the meniscus associated with the tetrahedron must break into rings, and no hysteresis is possible beyond the vapor pressure given by equation (*CC*). The dehydration curve is identical with the watering curve for values of p from zero to p_m; the absorption isotherm is completely reversible for values of p such that $0 < p < p_m$.

The effect of microscopic hysteresis is to alter the dehydration curve shown in figure 4a. The main meniscus will disappear at some point $0_{m'}$ on the dehydration curve instead of at 0_1, as shown in figure 4b; the water content is greater during dehydration, because of the presence of a number of saturated configurations not present in hydration, and by an amount indicated by the distance $0_1 0_{m'}$. As the vapor pressure is lowered the dehydration curve must run to the right of the hydration curve, but, as the saturated configurations disappear, approach closely to it. The saturated configurations become unsaturated at different vapor pressures, for they are in distribution depending on the porosity (the ideal soil is a random piling); and since the number of close tetrahedrons is small, deviations of the two curves should not be great near $\lambda_c = 19.5/r$. The dehydration curve must finally meet that of hydration at 0_m, the limit of

microscopic hysteresis. From there on, as the vapor pressure is decreased, the two curves are identical and are represented by $0_0 0_m$.

Figure 4b shows the complete cycle for capillary condensation in an ideal soil. $0_0 0_m 0_f 0_2 0_3$ is the hydration curve, and $0_3 0_2 0\ 0_m 0_m 0_0$ is that of dehydration. The existence of saturation hysteresis is clearly shown. The soil may exist completely saturated for any vapor pressure p such that $p_h < p < p_s$. The entire region of hysteresis is confined between the vapor pressure p_m for the junction 0_m of the two curves in the pendular region, and the vapor pressure p_s for 0_2 the junction in the saturation region. Both curves are identical in the

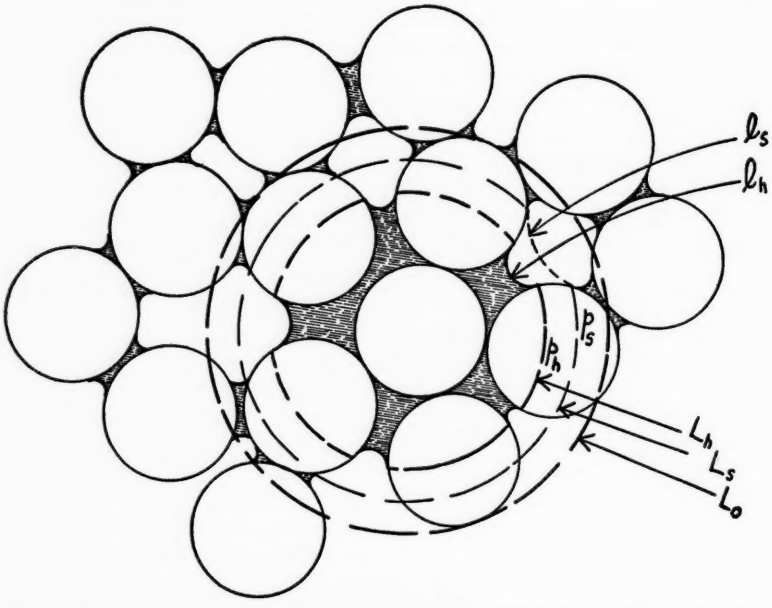

FIG. 5. SMALL PACKED SAMPLE WITH MENISCUS CONTRACTED TO THE INTERIOR OF THE PACKING

The notation used is identical with that of figure 3

region $0_0 0_m$ and $0_2 0_3$. In the hysteresis region there are two types of hysteresis, saturation and microscopic; the latter alone occurs when $p_m < p < p_h$, whereas both types may occur when $p_h < p < p_s$ (in the funicular region). On the curve $0\ 0_{m'}$, for example, the part of the packing over which the meniscus has passed contains the small saturated configurations characteristic of microscopic hysteresis while the remainder is saturated; on the corresponding curve for hydration $0_f 0_2$ the same configurations may be unsaturated.

In the saturation region ($p_h < p < p_s$), we may cross from the curve $0_m 0$ to $0_f 0_2$ and *vice versa*. Such a crossing would occur in a partially saturated pack-

ing. If we start at the point c on 0_m0, we recognize that the capillary-condensed liquid is in two distinct capillary states; first, in a bulk saturating part of the packing and bounded by the original capillary meniscus, now contracted to the interior of the packing; and secondly, as discrete masses existing in the remainder of the packing and formed as the main meniscus passed over the region; these relations are shown in figure 5. If we hydrate, both masses will grow; the main meniscus will merely adjust itself to the layer of grains where it happens to rest, and only a small amount of liquid will be absorbed, the process being similar to that on 0_20_3; the discrete masses will grow by condensation and, when the vapor pressure p_s is reached, will completely combine. In general, we should reach the curve 0_f0_2 at the point 0_2. The water content, for a given vapor pressure, along $c0_2$, in a packing which has been partially dehydrated to a point such as c and then re-hydrated should be greater than if the packing had been subjected only to hydration. On the curve $c0_2$ the water content is the sum of two bulks, one saturating part of the packing and the other composed of funicular masses embedded in the remaining part. The water content should be greater by approximately the difference, in the saturated portion, between the volume of the pore space and the volume of the funicular masses filling it at the same vapor pressure (Fig. 5). Hence, in general, the intermediate curve should meet 0_f0_2 at 0_2, the point of complete saturation. If, on the other hand, we stop hydration at some point d on 0_f0_2 and dehydrate, we then trace another curve de which represents the breaking up of the discrete masses which exist on 0_f0_2 at the point d (complete coalescence has not occurred, and does not occur, until 0_2 is reached). The curve de will not be identical with 0_10_fd because of microscopic hysteresis in the funicular region; many saturated configurations exist at d formed by prior coalescence and they preserve their forms during dehydration as long as possible. The curve de should finally intersect 0_m0_m0 at e somewhere near the boundary of the pendular and funicular regions.

The foregoing discussion of the absorption cycle has rested on the supposition that only the liquid and its vapor are present in the soil. Should a gas soluble in the liquid be present, the matter is more complicated, especially at vapor pressures near those of saturation. When the soil is hydrated, the mergence of funicular masses may occur in such a way that small bubbles of gas are trapped and held enclosed in the supporting grain configuration by the newly coalesced liquid. The gas, air for example, may remain thus enclosed for a long time. The hydration curve will be greatly modified near the saturation region (0_20_3, fig. 4b).

The presence of a meniscus disturbs the normal internal pressure of the liquid and must be considered (11). The pressure just over the meniscus is p, that in the interior less by an amount Δp where Δp depends on the curvature (Laplace equation). If the vapor pressure p over a funicular liquid mass, belonging to the hydration curve, for example, is increased, the curvature of the funicular meniscus must decrease; Δp then decreases and the interior pressure increases toward p; thus gas enclosed in the funicular liquid mass will be compressed and

tend to go further into solution. After complete coalescence has occurred and a continuous meniscus has formed at the saturation pressure p_s, the process will continue until the normal vapor pressure p_0 is reached. The hydration curve will be distorted; at some point a on 0_f0_2 the actual curve will leave 0_f0_2 and follow a curve $a0_3$ (broken line in figure 4b) instead of $a0_20_3$. The actual curve may not, in some cases, even meet 0_20_3. The reverse process accompanies dehydration. If the vapor pressure p over the meniscus is decreased, then the curvatures and Δp also will increase, with the result that the interior pressure becomes less; gas dissolved will come out of solution, and the soil will contain many microscopic gas bubbles, the volume of which depends on p. The soil, although apparently saturated, is not saturated by the volume of gas released in the packing. The normal dehydration curve 0_30_20b becomes replaced, actually, by the curve 0_3b (marked by a broken line in figure 4b).

If the soil sample is sufficiently large, the distribution of the capillary-condensed liquid will be influenced by gravity and it will no longer be homogeneous. If, however, the vapor pressure is known at one single point of the packing, the distribution can be calculated by a simple extension of methods given in another paper (8). If, for example, the vapor pressure p_A is known for a point A at the top of the soil, that at a point H distant from A by h cm. is[4] $p_H = p_A e^{(D_0gh/p_0)}$. It is then possible to determine the capillary state at any point in the soil in terms of the single vapor pressure p_A and the distance h. Since $\log_e (p_H/p_0) = \log_e (p_H/p_A) + \log_e (p_A/p_0)$ we have from equation (G)

$$\lambda = -\frac{\rho p_0}{\sigma D_0}\left[\frac{D_0 gh}{p_0} + \log_e \frac{p_A}{p_0}\right] \qquad (DD)$$

to be used in equations (M) to (CC) when determining the capillary state and quantity of liquid present in the soil. It is easily seen that all three zones of capillary distribution may be present if the soil column is of sufficient length. The vapor pressure at the reference point A and the length of the soil column determine which capillary zones of distribution are present. The problem is identical with that discussed in a paper (8) on the distribution of liquid in soils. The long soil column may be regarded as a superposition of a number of small samples each with a different vapor pressure; the distribution in each sample is homogeneous but varies from sample to sample. The distribution for an infinitely long soil column is identical with that shown in figures 4a and 4b. Figure 6 shows the relative retention represented as a function of the vapor pressure and also as a function of the distance h of a given soil lamina above a plane liquid surface. In an actual soil only part of the distribution there shown will be found, and the appropriate part of the curve will depend entirely on the range of vapor pressures prevailing in the soil. Figure 4b gives a more correct

[4] From the relation $h = -\int_{p_A}^{p_H} D\, g\, dp$ and from Boyle's law $D/D_0 = p/p_0$ we find $\log_e (p_H/p_A) = D_0gh/p_0$, from which is found the value given above; h is measured downward from the line $p_A = \text{const.}$

picture of the complete distribution, since disturbing effects such as microscopic hysteresis are shown.

So far, the discussion has been restricted to capillary condensation in the ideal soil. But, in general, both capillary condensation and surface adsorption are present. One, however, may be negligible in comparison with the other; the grain size is the controlling factor. With coarse soils composed of larger grains, capillary condensation is sufficient to account for the absorbed liquid. When, however, the grains approach colloidal size, surface adsorption cannot be neglected and can, especially at small vapor pressures when capillary condensation is negligible (very small rings), account for the greater part of the absorbed vapor.

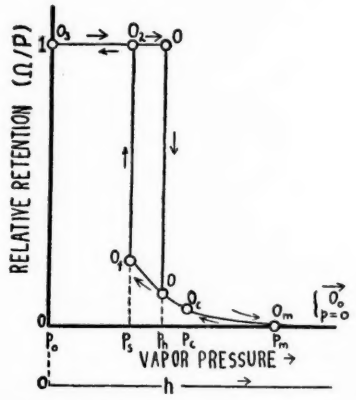

FIG. 6. SCHEMATIC DIAGRAM OF THE POSSIBLE MOISTURE DISTRIBUTIONS IN A LONG SOIL COLUMN

The relative retention is represented as a function, first, of the vapor pressure prevailing in a given lamina; and secondly, of the distance h of the lamina from a free liquid surface (the notation is identical with that of figure 4).

Surface adsorption occurs only on the parts of the grain surface not covered by capillary condensed liquid; for example, there is no surface adsorption in the saturation region, represented by $0_2 0_3$ of figure 4b. It occurs principally in the pendular and funicular regions. It will, where appreciable, displace the curves $0_0 0_m 0_c 0_f$ and $0_m \cdot 0_m 0_0$ of figure 4b to the right; the total retention is the sum of the capillary condensed liquid and the amount held by surface adsorption. It is, for a single grain, equal to the area of a sphere-surface, $4\pi r^2$, less the total area of all the polar caps of the grain covered by rings. The area of a polar cap is $2\pi r^2(1 - \cos\theta)$ (figure 7) where θ is the angle of the ring. If there are n contacts per grain and each supports a ring of liquid, the net area b will be given by

$$b = 2\pi r^2[2 - n(1 - \cos\theta)] \qquad (EE)$$

We have shown elsewhere (6) that $n = 6(1 + 1.828\,x)/(1 + 0.414\,x)$ where $x = (0.476 - P)/0.217$; from equation (H) we have $\cos\theta = 1/(1 + 1/r\lambda)$; hence, with λ given by equation (G), the surface available at any particular vapor pressure in the pendular region can be calculated. The total surface B_p available in 1 cc. of packing is $B_p = mb$ where m is the number of grains per cubic centimeter and given by $m = (1 - P)/[(4/3)\pi r^3]$ or the equivalent expression $m = (1 + 0.414\,x)/8r^3$. To find the total surface adsorption we must know

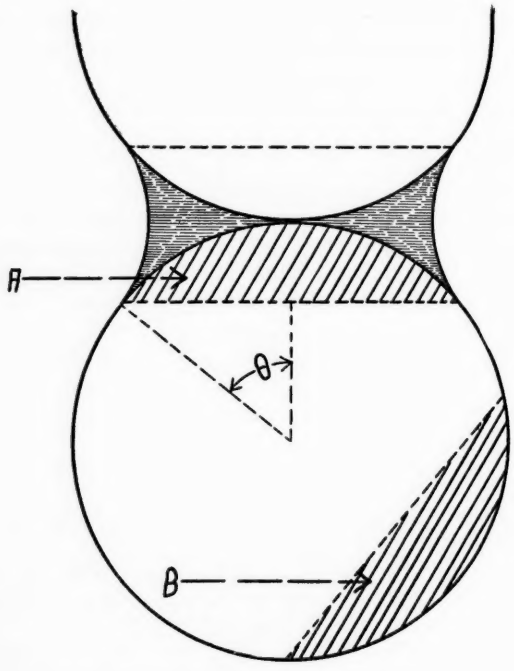

FIG. 7. SORPTION PHENOMENA ON A SINGLE GRAIN

The shaded areas A and B show portions of the sphere-surface covered by capillary-condensed rings; the remainder of the grain surface is available for surface adsorption (θ is the angle defining the size of the ring covering the segment A).

the concentration c (mass of vapor adsorbed on unit area) on the surface for the particular vapor pressure and temperature in question. The total liquid ω_p held by surface adsorption will be given by

$$\omega_p = cmb \qquad (FF)$$

We may proceed similarly in the funicular region, if, as discussed earlier, minimum capillary condensation is involved. The area b available for surface

adsorption on one grain is given by equation (*EE*). For the average packing used in the funicular region (6), however, $n = 12$; $\cos\theta$ is, from equation (*Q*), given by $\cos\theta = q/[1 + 1/(r\lambda)]$, with a value of q obtained from equation (*R*) and λ from equation (*G*). The total available surface per cubic centimeter of packed space is $B_f = mb$ where $m = (1 - P)/[(4/3)\pi r^3]$, and the total adsorption in terms of the concentration c is

$$\omega_f = cmb \qquad (GG)$$

The total retention Ω_p', for the pendular region, is the sum of the surface adsorbed (ω_p) and capillary condensed (Ω_p) vapor, obtained respectively from equations (*FF*) and (*M*), or

$$\Omega_p' = \omega_p + \Omega_p \qquad (HH)$$

Similarly for the funicular region, we have

$$\Omega_f' = \omega_f + \Omega_f \qquad (II)$$

where ω_f and Ω_f are, respectively, the actual amounts of surface-adsorbed and capillary-condensed liquid in the funicular region.

It appears from this discussion that capillary condensation is, for most ideal soils, chiefly responsible for the sorbed liquid. Surface adsorption is important and need be considered only when the grains approach colloidal size (10^{-6} cm.). A simple calculation will show that this is the case; we regard the adsorbed water as spread on the available grain surface at a thickness of one or two molecules and at unit density or even double that value. Actual soils are a mixture, and the predominant sorption process will be determined by the amount of colloidal matter present.

If the soil material is transparent, and is to absorb the vapor of a clear liquid, the optical behavior of the system is an important phenomenon. If the refractive indexes of the solid and liquid are close together, we may, as long as the soil is completely saturated, expect transparency. When, however, there is an unsaturated condition and rings or funicular masses of liquid are present, diffuse reflection from the capillary surfaces of such masses, especially those on the boundary, ought to render the soil opaque. Interference phenomena, no doubt, occur with soils composed of small grains. There should thus be a sharp optical transition on the dehydration curve, when, at the critical vapor pressure p_h, the distribution changes from saturation to one of discontinuous masses; the soil changes from a state of transparency to one of opacity. As the vapor pressure is lowered, the opaqueness should gradually disappear and the soil should be completely transparent when dry at $p = 0$. There should be a second transition, for hydration, when, at the critical vapor pressure p_s, the reverse change takes place; the soil should become gradually opaque and

then transparent when $p > p_s$, unless the saturated state is disturbed by the presence of a large amount of free gas.

The process of sorption, it is believed, is responsible for the slow transfer of soil moisture; a distillation or condensation takes place, generally, from or on the existing capillary liquid masses until the requirements of the Kelvin relation are satisfied. In that case, definite vapor pressures ought to be associated with the equilibrium of a long soil column. Table 1 shows the magnitude of the various vapor pressures to be expected for the sands used by King (4, p. 85–95; 8) in his retention experiments. Equation (*C*) has been used to compute these results; the necessary formulas can be established by following the procedure used for equation (*D*) of the text. These sands are comparatively coarse, and the vapor pressures differ but little. It must be remembered that these vapor pressures are for the final equilibrium state and that the time taken by a process of condensation in attaining them may be considerable.

TABLE 1

*Summary of vapor pressures associated with King's experiments**

	SAND NUMBER				
	20	40	60	80	100
Porosity	0.389	0.401	0.408	0.406	0.398
Radius of grains (cm.)	0.02373	0.00924	0.00776	0.00592	0.00413
$(p_0 - p_s)$ dynes/cm.^{2}	0.167	0.415	0.484	0.638	0.949
$(p_0 - p_h)$ dynes/cm.^{2}	0.233	0.568	0.656	0.870	1.310
$(p_0 - p_c)$ dynes/cm.^{2}	0.251	0.642	0.762	1.002	1.436
$(p_0 - p_m)$ dynes/cm.^{2}	0.754	1.935	2.305	3.022	4.330

* Water is used in these experiments; $\rho = 1$, $\sigma = 72$ dynes/cm., $p_0 = 1.27$ cm. of Hg, $D_v = 1.274 \times 10^{-5}$ gm./cm.^{3}

The literature, finally, contains, as far as can be ascertained, no record of experimental studies of sorption in ideal soils composed of larger grains; that such should be made is desirable. The glass pearls used by Green and Ampt, also by Haines (2) furnish a realizable ideal soil. With the technique used by McBain, Anderson, Patrick (5), and others in their work on the gels of silicic acid, experimental studies of sorption cycles in an ideal soil ought to offer little difficulty.

APPENDIX I

Equation (*A*) is obtained as follows: We suppose the liquid and its vapor in a closed system to be in equilibrium and consider a capillary surface in communication with a free liquid as in figure 8. We let p_0 be the vapor pressure just over the free liquid, p_a and $p_{a'}$ the pressures just over and under an element dS of the capillary surface (a and a' are respectively points immediately above and beneath the element); h is the height of the element dS above the free

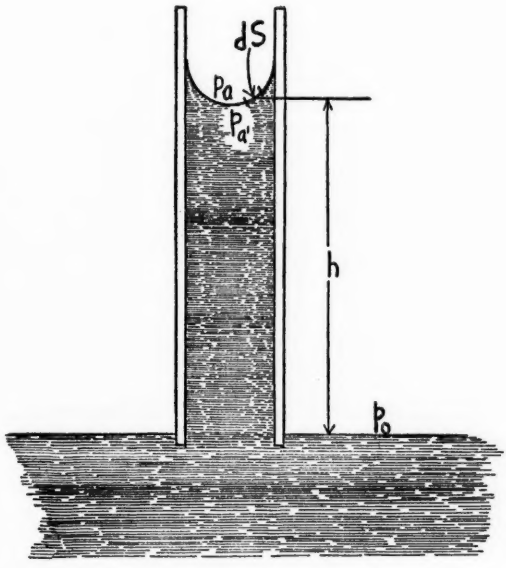

FIG. 8. SIMPLE CAPILLARY TUBE USED TO DERIVE EQUATION (c) OF APPENDIX I

liquid of density ρ and surface tension σ. We have then, if D is the density of the vapor at the height h, and g the acceleration of gravity,

$$p_0 = p_{a'} + \rho gh$$

$$p_0 = p_a + \int_0^h D\,g\,dh$$

If we set $\Delta p = (p_a - p_{a'})$, we find

$$\Delta p = \rho gh - \int_0^h D\,g\,dh$$

Now $dp = -Dgdh$ and $h = -\int_{p_0}^{p} dp/gD$; also, Boyle's law being assumed, $D/D_0 = p/p_0$; hence we find $-\int_0^h Dgdh = (p - p_0)$ and $h = -(p_0/gD_0)[\log_e(p/p_0)]$ where p_0 is the vapor pressure and D_0 the vapor density just over the free liquid. Therefore

$$\Delta p = -(\rho p_0/D_0)\log_e(p/p_0) - (p_0 - p) \qquad (a)$$

The Laplace capillary equation is

$$\Delta p = \sigma(1/R_1 + 1/R_2) \qquad (b)$$

where R_1 and R_2 are the principal radii of curvature of the element dS of capillary surface. Hence from equations (a) and (b) we have finally

$$\sigma(1/R_1 + 1/R_2) = -(p_0 - p) - (\rho p_0/D_0) \log_e(p/p_0) \qquad (c)$$

Equation (c) is sufficiently general for most purposes and can be shown, by thermodynamical considerations, to hold for a capillary surface not communicating with a free liquid, just as Kelvin has done (8). It is applicable to either a concave or a convex surface. If, for example, we consider a simple capillary tube immersed in a liquid, its radius, to a first approximation, is easily found; from equation (c), if R is the radius of the tube, we have, since $R = R_1 = R_2$

$$R = (2\sigma D_0)/\{\rho p_0 \log_e(p/p_0) - D_0(p_0 - p)\}$$

which is the equation derived by Anderson (1) also by Hückel (3, p. 237). If $(p - p_0)$ is negligible, as is generally the case (3, p. 237), we have the usual form of Kelvin equation

$$\sigma(1/R_1 + 1/R_2) = -(\rho p_0/D_0) \log_e(p/p_0) \qquad (d)$$

From equation (c), if p does not differ much from p_0, $\log_e(p/p_0)$ may be replaced by $(p - p_c)/p_0$ and we find the form originally given by Kelvin (10)

$$\sigma(1/R_1 + 1/R_2) = \frac{(\rho - D_0)}{D_0}(p - p_0) \qquad (e)$$

It is not difficult to obtain an integral form of equation (c), which is often more convenient for approximate calculation. We may multiply both sides of equation (c) by $dS \cos\phi$ and integrate; we obtain equation (A) of the text.[5]

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[5] For the partial integration $\int (1/R_1 + 1/R_2)\, dS \cos\phi = \int \cos\theta\, ds$, see any treatise on capillarity, e.g., Weber u. Gans, "Repertorium der Physik."

EROSION CONTROL WITH THE S.E.R.A.

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In the spring of 1935 a study of soil erosion and its control was undertaken as a work relief project by the Monterey County branch of the California Emergency Relief Administration, with the writer as project supervisor.[1] The work consisted of two phases: first a survey of erosion conditions in the supervisorial district chosen for the project, and secondly, the setting of plants and the placing of structures designed to control or reduce erosion.

EROSION SURVEY

The survey was made by 2-man crews whose members were chosen on the basis of their knowledge of the locality, ability to make reports, etc. A total of 325 farms were visited, representing about one-third of the area of the district. Of the rest, some land was essentially level and some in a limestone section, neither type of land presenting a serious erosion problem. The soil of the section studied is prevailingly sandy, some wind-borne, some residual from soft sandstone bedrock.

[1] For this work a nominal crew of 21 men was allotted, and a period of 8 weeks was allowed. The Monterey County Supervisors acted as sponsor for the project, providing office supplies, assessor's maps of the district, transportation for the project supervisor, etc.

The workers themselves were local men (the typist, a man or a woman) of varied ages and background, but mainly with agricultural experience. The compensation was based on a budget established individually for each worker and determined by his needs for rent, food, etc. Generally speaking, the man with the bigger family had a larger budget. The man was given enough work (in a 4-week month) to earn the amount of his budget. If, for example, his budget was $42 and the rate of pay $.50 an hour, his "work-ticket" would call for 84 hours' work, or 14 6-hour days. The work week was 5 days. Thus it was not uncommon, but rather the rule, that a man did not work the entire month (20 days). A man might be laid off for a week or two just as he had become well trained or demonstrated his fitness for the work in hand. These irregular labor conditions, however, caused no serious interference with the progress of the work.

Credit for the successful prosecution of the project must be ascribed largely to the interest and industry of the members of the crew, especially to two successive straw-bosses, Opel Wood and S. S. Gordon, of Aromas. Reports on the work done were prepared by S. S. Gordon and by Cyril Kirby. The project supervisor acted chiefly as a catalyzer. Grateful acknowledgment is also made of helpful suggestions given by Watsonville and Santa Paula members of the U. S. Soil Erosion Service (now known as the Soil Conservation Service).

Excluding the supervisor's time, the prosecution of this project cost 535 man-days of work distributed as follows: survey, 78; control measures, 441; typist, 16.

The chief object of the survey was to ascertain the magnitude of the erosion problem in the district. A summary of the information obtained is presented in table 1. Discrepancies in the totals of this table are due to lack of replies on the part of some of the farm owners or renters.

About one-seventh of the farms visited reported a reduced use of the land because of erosion. In many cases this reduction consisted merely in untilled patches left in the cultivated fields because of the difficulty of running plows and cultivators across gullies or because of the risk of causing further erosion. In a more aggravated stage, cultivated land was abandoned, wild plants were allowed to grow up, and the area was used for pasture or even left unutilized in brush.

In the case of reduced crop yields, it was obviously impossible to separate reductions due to erosion from those ascribable to other causes. For about

TABLE 1

Summary of information obtained in erosion survey, District 1, Monterey County, California, February–April, 1935

Amount of erosion and control

No erosion	121 farms	Control practiced by owner	86 farms
Little erosion	133	Control by owner unnecessary or neglected	224
Much erosion	65		
Total	319	Total	310

Land use reduced because of erosion	47	farms
Land use not reduced by erosion	266	
Total	313	
Crop yields less than earlier yields	158	farms
Crop yields not notably less than in former years	119	
Total	277	

half the farms visited, lower yields were reported, but the most we can say is that in some of these instances soil erosion was undoubtedly the main cause.

The small amount of clayey soil in this section is found mainly in level patches and is thus not subject to washing; but where it occurs on a moderate slope the erosion, as would be expected, is less than on a similar slope with sandy soil.

In the survey the land was characterized as steeply sloping, moderately sloping, or level. The amount of vegetative cover was also noted. The information obtained does not indicate much correlation betweeen these factors and the amount of erosion. Most of the farms studied were situated on moderate slopes. Erosion was reported as varying from none to considerable on these areas, both bare and with good vegetative cover. In the relatively few cases where a timber or brush cover was noted, however, erosion was re-

ported to be inappreciable. On the other hand, one or two farms situated on steep slopes with sandy soil were reported to be relatively free of erosion.

For the purpose of determining suitable plants for vegetative control of erosion, a note was made of the important native vegetation on each farm visited. The designation "native" must be given a very broad interpretation in this connection, for the three species most commonly observed are all naturalized European plants; namely, filaree (*Erodium circutarium* and perhaps *E. moschatum*), mustard (*Brassica campestris*), and wild radish (*Raphanus sativus*). Filaree, a rosette plant in youth, belongs to the geranium family, and the other two are fast-growing members of the mustard family. All three are annuals (the radish may also be a biennial). These luxuriant crops are reputed to afford excellent protection to the soil (either as volunteers or sowed), but were not usuable in the S.E.R.A. work. Filaree, incidentally, is a valuable forage plant. On abandoned land coyote brush (*Baccharis pilularis*, Compositae) is one of the first invaders. It would undoubtedly be useful on raw slopes, but there is a local prejudice against it as a troublesome weed. In orchards its presence is regarded as a sign of neglect.

Certain current agronomic practices have a good deal of bearing on the erosion problem in this section. The extensive raising of peas, for example, promotes soil losses. In the past, when oats were more commonly raised in this section than now, the ground was kept fairly well covered, and cultivation was not practiced after sowing. Peas, however, now a more profitable crop than oats, are spaced farther apart and cultivated after each rain. The soil is thus left in ideal condition for eroding when the land has any appreciable slope. Again, it is locally considered good practice, because of the dry summer season, to keep the ground bare under and between orchard trees at this time so that valuable moisture will not be lost through transpiration by other plants. As a result, considerable erosion occurs in the orchards of the section with the advent of the fall rains or when a late spring rain follows spring cultivation.

Undoubtedly changes in farm management would result in an astonishing improvement in the erosion situation in this section, as in many other agricultural regions. It is also unquestionably true that some of the land here is bound to erode under almost any system of cultivation and should never have been cleared of its native growth in the first instance. That, however, is more easily observed than remedied. There are places where the use of other crops than the present ones would be far better for the farmland, but the other crops do not bring in satisfactory returns. One promising ground-covering crop, however, is worth mentioning, namely eucalyptus. There are several groves of these trees in the district, located on ridge tops where the sandstone bedrock is near the surface and cultivation is utterly out of the question. Harvested at the age of 15 years for fuel wood, this is said to be a surprisingly profitable crop, comparing very favorably, on the basis of annual returns, with other crops of the region. The wood seems to be well liked for

cooking and heating stoves. The second crop is a coppice stand, which, if properly thinned early in life, will yield an appreciable amount of pole, post, or fuel wood.

Besides the deterioration of agricultural land, soil erosion has another very important aspect, namely, the blocking of roads by sand carried down from the adjacent slopes. It is this phase of the problem which is of most concern to the county engineer. Sand removal is a costly annual item. Unfortunately the remedy is not to be found in such a program as ours, but rather in improved agricultural practice. Sometimes faulty location of a minor road is responsible for the troublesome sand which washes down to a more important road.

CONTROL MEASURES

The efforts made to control erosion were admittedly of an experimental nature, but were based on the teachings and practice of the U. S. Soil Erosion Service, modified by local conditions and experience. We were well aware in this work that we were attacking only the middle phases of the problem. On the one hand, we were not in a position to do much about the insidious sheet and shoestring erosion, which, though less spectacular than the familiar gully erosion, is none the less serious in reducing the value of agricultural land. The control of such erosion is mainly a matter of farm management. On the other hand, we were unable to install any of the more elaborate control structures of rock, masonry, concrete, or timbers, such as the Soil Erosion Service is building on its experimental areas. The object of the S.E.R.A. work was to pay *wages*, and money for *materials* was accordingly limited in amount. There is a question, too, as to whether the requisite skilled labor would have been available on the local relief rolls, even if it had been possible to obtain more materials. As it was, most of our work was done with materials which could be had for the asking, largely willow from the stream bottoms and the similarly ubiquitous haywire.

The following control measures were undertaken:

Planting of willow cuttings and sea-fig (*Mesembryanthemum aequilaterale*, locally known as "beach-apple").
Construction of nearly 200 soil-saving and check dams.
Construction of half a dozen "brush stairways."
Placing of brush on eroding slopes and in narrow gullies and at gully heads.
Laying of 180 feet of 12-inch concrete drain pipe.
Setting of sand bags.
Digging of diversion ditches.

Several variant types of dam were tried, mostly constructed of willow in some form or other. In a few cases small logs, up to about 6 inches in diameter, were wired to upright willow posts and the ends set well into the bank. Although these proved fairly effective, especially for relatively high dams in narrow gullies, they made such heavy demands upon the available material that it was impracticable to employ them on an extensive scale.

Employing smaller material is the wattle (or woven) dam, a type of structure to which willow is admirably adapted. For these dams, also, willow posts were used. These two dams are essentially the same as those pictured by Ramser.[2]

Another type was developed by the crew in the course of the work. This consisted of a double row of posts with brush between. The posts were spaced 1½ to 2½ feet in the row, with the rows a foot or two apart, mostly 15 or 16 inches. The brush was tramped down; the posts were wired together in pairs, the wire extending across the brush; and finally the posts were driven deeper to compact the brush. The result is a sort of brush wall 2 to 4 feet high, mostly 3 feet. Presumably this kind of dam has been used elsewhere, but A. B.

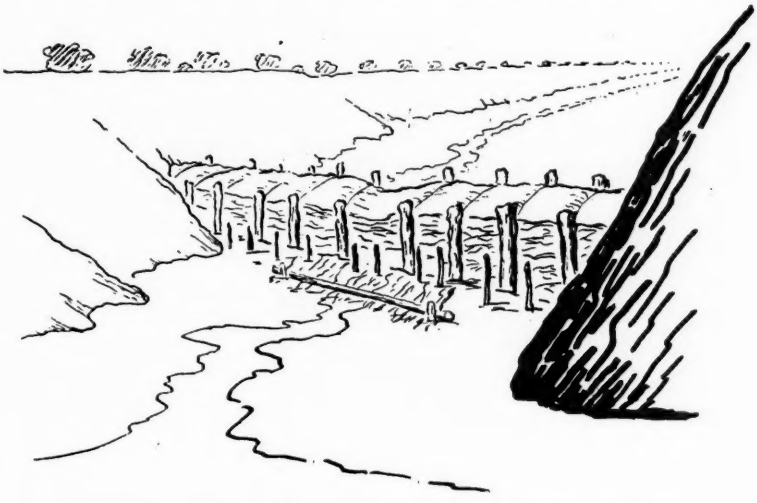

FIG. 1. "HARRIS" TYPE DAM IN PLACE, WITH APRON AND ROW OF CUTTINGS ON DOWNSTREAM SIDE

Harris was the originator of it on our work, and his fellows referred to it as the "Harris" dam. It seems to be a very effective model and in our later work was used to an increasing extent. It is illustrated in figure 1.

Another kind of brush dam was made with a broad base and narrow top. After the brush was piled in a windrow across the gully, pairs of posts or stakes were driven into the ground at an angle, one on the upstream side, the other downstream. Where these crossed above the brush, like saw horse legs, they were wired together and subsequently were driven deeper, thus compacting the brush mass. From its appearance we spoke of this type as the "sawbuck"

[2] Ramser, C. E. 1925 Gullies: how to control and reclaim them. *U. S. Dept. Agr. Farmers' Bul.* 1234: 15, 17.

or "rat's-nest" dam. It proved to be one of the most effective kinds for catching sediment.

A few dams were made of hog wire or chicken wire fastened to redwood pickets or willow posts.

In some of the smaller gullies, brush of various sorts, thoroughly tramped in, was used to good advantage. With brush of smaller caliber, stakes were used to keep it in place. In narrow, steep gullies, these brush fills sometimes took the form of crude dams with bare spaces between the dams.

A further development of this control method was the "brush stairway," as the men aptly called it. This type of structure was employed in some of

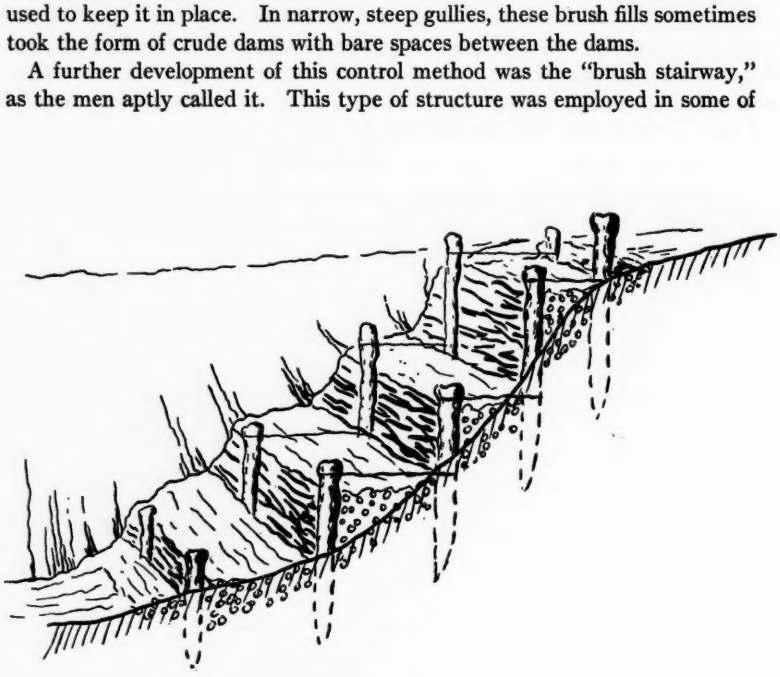

FIG. 2. "BRUSH STAIRWAY" IN NARROW, STEEP GULLY

the steeper gullies, often narrow laterals extending into cultivated land on the sides of an older, larger gully. It consists of masses of brush (of willow or of orchard prunings) placed in the gully one above another like a flight of steps, the front of each block of brush set against a line of posts deeply driven into the soil a foot or two apart. Sometimes the posts of one step were wired to those of the next step above. Depending on the width and pitch of the gully, the brush steps were made with treads up to 4 feet deep and 7 feet wide. The risers might be 2 feet in places. This structure is illustrated in figure 2.

When any obstruction is placed in a water channel there is the risk that the water will erode the earth around or under the obstruction. It is for this reason that the casual dumping into a gully of miscellaneous trash, such as

orchard prunings, old stoves, worn-out bedsteads, and wrecked automobiles, often proves nearly or quite valueless in checking erosion. All dams and other barriers should preferably be set well into the walls and bottom of the gully and should be lower in the middle than at the ends. In the case of a dam with vertical face, it is essential to provide some sort of an apron to prevent undercutting by the falling water on the downstream side. In our work we used for this purpose willow branches laid parallel to the direction of water flow, adjacent to the lower face of the dam, and held in place with a cross pole, as recommended by Ramser[3] (fig. 1).

The planting consisted mainly of willow cuttings. These were of various lengths and diameters, running 3 or 4 feet long set with half a yard extending above ground. Some were as much as 2 inches in diameter. Where willow twigs were available in quantity and the soil permitted, the cuttings were set a foot or so apart just below a dam, or sometimes both above and below (fig. 1). The posts of the dams were also usually of willow 2 or 3 inches in diameter.

Examination in the fall of 1935 disclosed a gratifying amount of new growth on willow cuttings, posts, and even some of the horizontal wattles. In a few cases willow posts bore sprouts 6 or 8 feet tall (in October). In some instances sprouts on posts and cuttings had died after reaching a length of a yard or so. It is to be assumed that there was a corresponding root development, which of course would help to hold in place the sand of the gully bottom. Possibly, too, the roots are still living and will send up new sprouts when the rains begin. In one large gully, 10 or 20 feet deep and 40 to 75 feet wide, the cutting of willows for dam construction resulted in a dense growth of sprouts 2 or 3 yards tall in the spaces between the dams.

In a few places sea-fig was used. Although this rapidly growing, decumbent, native succulent (of the family Aizoaceae) is employed locally to a considerable extent on cut banks along the roads, some farmers object to its use in cultivated fields because of a fear that it will spread as a troublesome weed. Judged by the apparent ease with which it is kept under control adjacent to cultivated orchards in some parts of the district, this apprehension is probably groundless. Nevertheless it is something to be reckoned with in planning control work.

On one particularly bad gully with slipping sides, both slopes were planted with sea-fig cuttings a foot or two long spaced one foot apart. By the middle of August these cuttings had made satisfactory growth, with an encouraging percentage of survival.

Since there was no publicly owned land available for our experiments, the control work was done on private land after the owners' permission had been obtained. As a general policy, there is definite objection to spending public money for projects benefiting only a few individuals. In view, however, of

[3] Ramser, *l. c.*, p. 15.

the need for experimentation and the educational value of the work, the use of private land was considered warrantable in this instance.

On the whole, the work done can be regarded as successful. The dams and brush fills have for the most part caught water-borne sand; willow cuttings and posts have sprouted and unquestionably developed fairly extensive root-systems; sea-fig is growing well; and the brush fills seem to favor the growth of blackberries and other vines. Before final judgment is passed on the effectiveness of the measures taken, at least a year should be allowed to elapse. It is to be kept ever in mind, too, that such work cannot be done once and for all and then left to itself. The control of erosion requires constant watchfulness, and control structures must be kept in repair and augmented as needed. This is not an S.E.R.A. task.

Although the primary purpose of the project was to provide relief work, yet it was hoped that our activities would serve to stimulate interest in soil conservation in Monterey County as in the case of the large-scale demonstration project of the Soil Erosion Service in adjacent Santa Cruz County. To what extent this has been true is impossible to say. Unquestionably our work had considerable effect which it would be hard to measure quantitatively. In one case a survey crew had almost completed the preliminary interviewing and took the supervisor to the farm to make final arrangements for doing a piece of control work. Upon reaching the site we were much surprised to find the work well under way along the lines suggested by the survey crew the day before. The owner had not waited for help from the S.E.R.A. crew. Again, an inexperienced farm operator seemed to welcome our work not merely because of the structures we installed on his property, but because of its value to him as a pattern for further work on his ranch. Yet another owner, on his own initiative, installed a structure supplementary to the S.E.R.A. work, before we had finished with his property.

SUMMARY

With a crew of S.E.R.A. workers a survey was made of erosion conditions in one supervisorial district of Monterey County, California, and experimental control measures were undertaken.

The results of the survey (which covered one-third of the area of the district) afford little basis for correlating erosion with soil, slope, or cover conditions, except that with a timber or brush cover erosion was inappreciable. On more than half the farms visited, erosion was noted, serious erosion on one-fifth of them. On one-fourth, some sort of control was practiced by the owner. On one-seventh, land use had been reduced because of erosion. Ill-advised farm management is responsible for much of the erosion observed.

The control measures included building of approximately 200 dams and other structures, largely of willow; planting willow and sea-fig; laying drain pipe; and digging diversion ditches. It was not possible within the limitations of an S.E.R.A. project to undertake a complete treatment of the erosion problem in the district, but the measures taken appear to be effective as far as they go.